

Measured Sense on Pollution

SIR ERIC ASHBY has been the chairman of the Royal Commission on Pollution for the past three years and has frequently been criticized for not having used his position as a means of amplifying popular discontents about disposable bottles and other contemporary nuisances. His critics owe themselves the duty of reading his address this week to the Royal Society of Arts, one of the most moderate statements of what needs to be done and how it should be done, so far to have appeared. Whether the Royal Commission itself will and should long survive Sir Eric's impending departure—he retires at the end of the month—is doubtful. The need for the Royal Commission was, to begin with, a reflexion of the British government's wish to have some guidance as to how it should react to the great fuss that people were then making about the environment, and it has long since become apparent that British government departments are well equipped to take environmental problems in their stride. It would be much simpler if this were openly recognized, and if the exceptional device of a Standing Royal Commission were abandoned.

The theme of Sir Eric's lecture is that freedom from pollution costs money but that, in the present climate of opinion, people in countries such as Britain are prepared to pay quite substantial sums of money for clean air, clean water and public amenity. He argues that technological innovations must be assumed to have a positive social value in a first approximation, for otherwise they would not be taken up. But there follow a number of subsidiary questions to answer—are the side effects of technological innovation damaging to people and, even if they are not, may not society as a whole wish to be rid of these side effects? Sir Eric quotes the economists' view that everything would be simpler if the cost of technological innovation were included in the cost of manufacturing operations—one of the proposals canvassed but not adopted in the Royal Commission's third report—but goes on to describe how, at least in Britain, it may be more effective to persuade industrial enterprises to follow sensible courses, quoting the switch from stable to degradable detergents as an example. The fuss in the United States about the side effects of phosphates in detergents is, in his view, a cautionary tale. Fanned by public excitement and the mass media, the movement to ban detergents containing phosphates reached the point at which several states had adopted legislation on the subject and, by the beginning of 1970, Senator Edmund Muskie's subcommittee was expected to recommend a change of federal legislation enforcing a switch from hard detergents to materials based on nitrilotriacetate, but in no time at all Professor Samuel Epstein showed that the cure might be more dangerous than the disease. The moral is that public pressure is not an infallible guide to action. Sir Eric goes so far as to think that the legislation now being promulgated to restrict the emission from automobiles, in Europe as

well as the United States, may be similarly injudicious—"I believe we and the Americans have over-reacted". And if it is a question of how best to spend money to reduce the side effects of the motor car, may it not be preferable to do something to reduce casualties from road accidents?

In his lecture this week, Sir Eric Ashby went on to deal with the complex of issues raised by Professor Dennis Meadows and his team of colleagues at the Massachusetts Institute of Technology. He appeared to accept the validity of the arguments of those like Professor Barry Commoner who have said that gross pollution is a product of the size of a population, its consumption of goods and services and of the amount of pollution produced per unit of goods and services, and acknowledged that the time must come when it will be insufficient simply to seek to control the degree of pollution per unit of output—steps will also have to be taken to stabilize population and to restrict consumption. But he has no patience with *The Limits to Growth*, chiefly because "it omits, deliberately or by mere oversight, known data which, if they had been included, would have given quite unsensational predictions about the prospects for the condition of man". As such, "it deserves the censure of scientists". But what if the assumptions had been correct and if the conclusions had been startling? Sir Eric acknowledges, as do all sensible people, that there is a need that society should be able to respond rationally to changing circumstances. Moreover, it is important that people should keep closely in mind the overriding truth that the case against pollution is not at present that the side effects of modern industry are known to be damaging but that there is a proper

Nature Index

The combined index for *Nature*, *Nature Physical Science* and *Nature New Biology* for 1972 will be available at the beginning of May.

The 1973 index will be available in January 1974. Thereafter, a two monthly index of articles which have appeared in the *Nature* journals will be included in *Nature* soon after each volume is completed. The annual index, which will still be published, will be more comprehensive and will include details of everything published during the previous year in the three journals.

Copies of the 1971 index, which was published at the end of last year, are still available from the *Nature* Subscription Department, Macmillan Journals Limited, Brunel Road, Basingstoke, Hampshire, at a cost of £1.00 or \$3.00, postage paid.



wish among all kinds of people that freedom from nuisance, public amenity, is a commodity to be purchased for its own sake and for the benefit it will bring to later generations.

What does this imply for public policy, not merely in Britain but elsewhere? The first thing to be said is that because freedom from pollution must be regarded as a public good to be purchased with public funds, it is imprudent of societies to neglect to make comparisons with other kinds of public purchases—social security, education, health care and the like. One of the chief complaints against those who would have governments take draconian measures against polluters is that they have often neglected this comparison. Yet even in the affluent societies in which it is now understandable that people should put the purchase of public amenity high on the shopping list, there remain social needs of great importance which cry out for attention. In Britain, for example, investment in the National Health Service could well be held to be more beneficial than, say, further investment in clean air or clean water. To be sure, the choice is by no means clear cut, but the important point is that a choice of some kind must be made, at least if the government is at the same time required to pursue fiscal policies which minimize the manufacture of bank notes. The difficulty is that the population is rarely given a chance to make rational decisions for lack of sufficiently detailed and accurate information, which is why it falls to the government to guide discussion more firmly than in the past. Is it too much to ask that the British government should follow the American in providing the detailed costing of environmental policies which the Council on Environmental Quality has been publishing in recent years, and that it should also extend the techniques of cost/benefit analysis to other kinds of social investments with which improved amenity may be compared?

The question of how manufacturers and other enterprises which cause pollution should be regulated is also an economic question. Sir Eric Ashby is apparently persuaded that diplomatic negotiations between governments and polluters are sufficient, but such techniques are necessarily arbitrary. There is a tendency for governments to tackle the problems that can be tackled easily by administrative procedures and to let others slide away. In all the circumstances, it would be in the long run beneficial if some serious attempt were made to saddle polluters with the social costs which their activities entail—to internalize the externalities, as the economists are fond of saying. There are, of course, serious and almost philosophical problems in such procedures. Many people will protest that it is impossible to value numerically the absence of sulphur dioxide, for example, from the atmosphere. This, however, is too gloomy a point of view. There is no reason why governments should not operate a system of taxes which would, for example, be levied on such things as the total output of sulphur-dioxide from factory chimneys, with geographical variations from place to place designed so as to penalize most severely those who add to concentrations of pollutants which are already a nuisance. If governments were prepared to introduce taxes of this kind gradually, they would find that existing industries would not be seriously disrupted. At the same time, it would be possible to work towards a level of taxation which pro-

vided polluters with some incentive to be less of a nuisance and at the same time to estimate the consequences of the new taxation for the costs of their operations. What it would, of course, be necessary to avoid is a situation like that in which the government of Sweden found itself last year, when a proposal to levy a tax on disposable bottles turned out to be unacceptably expensive for consumers. But where major sources of industrial pollution are concerned, there is plenty of scope for constructive action so long as governments and their electors are willing to move gradually.

More Bad Science TV

THE complaint in *Nature* on February 2 (241, 300; 1973) that BBC television programmes have slipped into the bad habit of giving too much publicity to and too sympathetic an explanation of the irrational elements in modern science seems to have caused great indignation at the BBC. One member of the corporation, himself a distinguished and level-headed contributor to the corporation's broadcast output, said the other day that he and his colleagues would have protested in writing if the complaint were not so self-evidently wide of the mark. Others have said, with more justice, that a complaint like this should at least have acknowledged that the BBC has won a high reputation for itself in the presentation of vast themes in science to a popular audience by means of the outstanding television programmes jointly the work of Mr Philip Daly and Mr Nigel Calder, so that the redemption of the corporation's reputation for the level-headed presentation of science does not rely entirely on Dr Jacob Bronowski's impending series of programmes. Nobody will cavil about the high quality of these programmes, which are exceedingly distinguished but also, as it happens, infrequent—Messrs Daly and Calder manage one programme every eighteen months or so. And those who doubt the validity of the complaint that the majority of the BBC's routine science output on television is dangerously infected with heresy could do worse than look again at last week's *Horizon* programme, which came as close as any programme could to a celebration of irrationality.

The programme rejoiced in the title "Science is Dead—Long Live Science". It began with a re-run of some of the most gruesome of the gruesome films collected in recent years from Vietnam and with a facile and misleading analysis of the research and development budget of the United States government—what sense does it make to compare federal expenditure on military development with federal expenditure on basic research at universities without at least acknowledging that in the United States the bulk of civil development is paid for directly by the commercial companies who stand to benefit? It was entirely proper that the programme should have interviewed the two scientists originally responsible for the development of napalm and the defoliants which have been used in Vietnam, and that it should explain that both people are alarmed to see what use has been made of their research, but does it follow from that that science is dead? And even if it is the case that the United States forces in Vietnam had worked out ways of

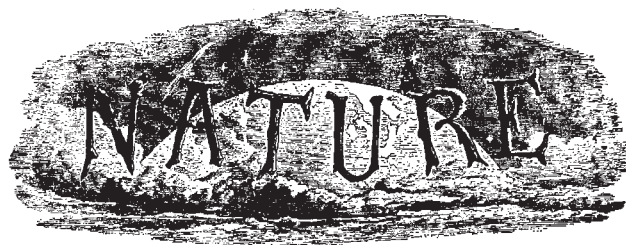
bugging the battlefields with hidden sensors, is there any rational case for implying, as the programme did, that the profession of science and not the political climate which made the Vietnam war possible is to blame? What those who devised this witless programme have done is to foster the entirely mistaken but popular belief that the Vietnam war would not have happened if it had not been for scientific research. There is, of course, an entirely valid case for saying that some of the horrors of the past few years have had their origin in the laboratories, but does anybody seriously think that there would have been no war if scientists had behaved differently or if the scientific community had been differently organized?

The rest of the Horizon programme was even more dangerously irrational. It is interesting that a scientist previously on the staff of the "national standards laboratory" (presumably a reference to the National Bureau of Standards) should have been so offended by FBI investigations of his political activities that he chose to resign and run a printing press instead, but the programme might have reminded those who saw it that there are many countries in which government employees are forbidden by their terms of employment to engage in any kind of public political activity and that there is a case for saying that scientists who dissent from government policies should not in any case elect to work in government laboratories. The Horizon programme also dealt with the notorious case of strip mining in West Virginia and with a young man who had given up his scientific career to battle against the bulldozers. But is it not simply bad reporting to fail to mention that one of the scandals of coal mining in West Virginia is that the operators are not required by law to restore the terrain they despoil (as they are in other parts of the world) and that one of the difficulties with which the environmentalists have to contend is illustrated by the way in which Mr Jay Rockefeller, who ran on an anti-strip mining ticket in the elections last November, was defeated presumably by the wish of the local population to put jobs before amenity?

The rest of the programme was largely an over-sympathetic account of a commune of young scientists who rejoice in the name "New Alchemists" and who appear to spend their time making windmills out of discarded differential gears, making compost and cultivating fish in tanks surmounted by greenhouses made with plastic strung across a geodesic dome. The commune's ambition appears to be the development of a life style that will provide a method of subsistence for similar communities when eventually the holocaust comes—and the Horizon programme used Dr Jonathan Beckwith not merely as an authority for saying when civilization will end but also as an excuse for alarming its audience with tales of genetic engineering. Quite apart from the muddled explanation of their activities which the members of the commune provided—the simple ecology of a fish tank was held to be incapable of objective analysis—the programme gave, no doubt by accident, a striking insight into the tendency of the New Alchemists to talk as if their secret knowledge would in due course allow them to ride omnipotently over the problems with which ordinary people contend. In the circumstances, perhaps, it is not surprising that they should have finished with a dash of Sun worship and the chanting of the strange word

"Om" around a camp fire. If the objective of the programme's producers had been to analyse an interesting if familiar tendency for groups of people to see how far they can succeed in living without the appurtenances of modern civilization, it might have been useful, but the reporting would have had to be sharper and less reverential. What, in practice, they have succeeded in doing is to construct a programme that might better have been called "Science is Dead—Long Live Unreason". Those who wish to defend the BBC against the charge of being anti-scientific in the bulk of its popular television coverage should do something to explain how this disastrous programme came about.

100 Years Ago



NOTE ON A POLYDACTYLOUS CAT FROM COOKHAM-DEAN

BY the kindness of Dr. Plumbe, of Maidenhead, I have been able to procure one of these cats; and from the many curious points he possesses, I think a note on his peculiarities will interest some of the readers of NATURE.

Readers of Mr. Darwin's "Origin of Species" are familiar enough with the illustration he gives of correlation of arrest of development in the deafness of blue-eyed cats. Some years ago I showed that our great naturalist had fallen into error on this point, and that the correlation is not between the blue eyes and the deafness, but between the latter and the sex of the cat.

I have made a great many inquiries on this point, and have completely confirmed my former observation, that all perfectly white tom-cats are deaf, and that they have blue eyes occasionally, because that item of beauty is common among white cats. I have seen many white Tabithas with blue eyes, but none of them were deaf. My little "Pudge" from Cookham is perfectly deaf, and has one blue eye and a yellow one. For the first few days after I had him, I thought he could hear a little, but am now quite satisfied that his deafness is complete, though he is alive to sounds conveyed through solid media. A further point of interest is that he is not mute as most deafs are, but there is a kittenish shrillness in his voice and a loudness in his purring, which are not commensurate with his age. I think, therefore, that it is possible that early in life he may have heard a little, for I know of two instances where perfect mutism accompanied the deafness in cats, and I do not know of any contrary condition. The one yellow eye favours my view that "Pudge" may have heard in infancy his mother's voice. His sense of touch is extremely acute compared to that of another cat I have, but his sight does not seem so sharp as that of cats generally is. He has twenty-six digits, and these are arranged—seven on each fore limb, and six on each hind limb. The supernumerary digits on the fore limbs are thumbs, and are placed one on either side of the true pollex, being joined to it, but having no metacarpal bones. In the hind limb the supernumerary digit is probably of the same nature, or a supernumerary index, being placed on the outer side of the hallux, and attached to the tarsus by a completely-developed metatarsal bone.

LAWSON TAIT

From Nature, 7, 323, February 27, 1873.

OLD WORLD

Select Committee Picks up the Pieces

THE Select Committee on Science and Technology was taken aback last week when Mr Michael Heseltine, Minister for Aerospace and Shipping, appeared before it and announced that the government had decided to scrap the hovertrain project which Tracked Hovercraft Limited have been running for the past five years at a total cost of about £5 million. At that meeting he announced that the company's work on linear motors would be continued through a joint arrangement between the National Research Development Corporation (NRDC) and Hawker Siddeley under which the government would subscribe £500,000 over four or five years and Hawker Siddeley £100,000. He also said that Tracked Hovercraft's work on magnetic suspension would be carried on by British Rail with the help of a £150,000 grant.

Later that same day he let it be known that there was a strong possibility that a joint company, involving the NRDC, Hawker Siddeley and British Rail, would be formed.

This week the select committee settled down to analyse the rights and wrongs of the decision and to decide whether or not it had been made at the right time. Appearing before it were members of the management of Tracked Hovercraft Limited and representatives of the company's staff association.

Fundamental disagreement with several of Mr Heseltine's reasons for cancelling the project emerged from the meeting. Mr T. G. Fellows, managing director of Tracked Hovercraft, criticized Mr Heseltine's view that there is no prospective market in Britain for the hovertrain, and that "the country would not tolerate the duplication of track" involved in building a hovertrain link between, say, London and Manchester. Mr Fellows pointed out that British Rail itself expected a capacity problem by the mid-1980s, when a British hovertrain might reasonably be expected to be a viable proposition, and would itself be "duplicating" track or looking for new routes altogether. As far as possible environmental damage is concerned, Mr Fellows said that a hovertrain track need not be completely elevated, but could be partly laid at ground level or in tunnels. And he added that noise gave "no grounds for concern".

Tracked Hovercraft also disputed Mr Heseltine's estimates of the journey time for a hovertrain on a route from London to Maplin Sands, the proposed

site for the third London airport. Mr Heseltine had said that a hovertrain would take 36 minutes to do the journey whereas the company estimate a time of about 20 minutes. Mr Fellows said that as he had understood the views of the Department of the Environment, British Rail's Advanced Passenger Train would form the first link in 1980 and the hovertrain would supplement it in the 1980s or 1990s. It would serve both as a showcase and as the first of a series of inter-city links.

Members of the staff association took Mr Heseltine to task for his lukewarm attitude to the prospects of European cooperation on research into high-speed ground transport. In its submission to the select committee the association says that the European commission "is expected to approve before this April, a policy for a network of routes for Europe including Britain" and that the commission has recommended that European governments should engage

in joint research into new transport media, notably tracked hovercraft. Mr M. R. Bailey contended that when Mr Heseltine had spoken of a lack of foreseeable demand in the home market, he had failed to take proper account of how bound up Britain now is with the rest of Europe.

Mr Fellows described what seemed to him to have been a change of heart within the Department of the Environment about the hovertrain at the end of last year. Before then, he said, there had been a good prospect of his company combining resources with British Rail to their mutual advantage. Mr Bailey suggested that because of the troubles with the Advanced Passenger Train, for example with the gas turbine propulsion system, the suspension system and the general structure, the Department of the Environment no longer has money available to fund the contracts which Tracked Hovercraft needed.

ENERGY POLICY

Robens's Solution

AN independent Energy Commission to advise the government on energy policy was called for this week by Lord Robens, former chairman of the National Coal Board and now chairman of Vickers.

Lord Robens said that the commission should be a permanent body staffed by experts in fuels economics and in the specialist technologies. It should undertake comprehensive long and short term studies and its remit should include coal, gas, electricity, oil and nuclear power.

"The commission, having no particular energy axe to grind would be able to objectively produce an energy policy, planned entirely in the national interest . . . the in-fighting between the publicly owned energy industries would cease, as their place in the energy pattern would be properly established".

The resources of the Department of Trade and Industry are totally inadequate to do the task, according to Lord Robens. Attacking the 1967 White Paper on energy, which he described as a miserable failure, Lord Robens pointed out that the civil servants responsible for it "are no longer there to face the consequences of its impotence". A permanent com-

mission would be able to accomplish more than any *ad hoc* body.

Lord Robens, who was delivering the Potterton Lecture to the Institution of Heating and Ventilating Engineers, said that "without the slightest doubt, energy resources over the next ten years are going to be sorely stretched." He pointed out that "to an advanced industrial economy such as ours, the importance of secure energy supplies is out of all proportion to the cost . . . short term commercial price considerations should not be allowed to obscure the very real need for security of supply".

The commission would resolve the questions of who is to carry spare capacity and stocks, of how competition between indigenous fuels can be limited so the national interest is best served, and of how the commercial "rules" under which energy interests operate should be re-assessed in order to give a greater weight to long term considerations.

If the government struggles on to produce an energy policy from its own resources it will surely fail as miserably as it failed in the energy white paper of 1967, Lord Robens said. "Only an independent commission, in my view, would get the juxtaposition of coal, gas, electricity, oil and even nuclear energy about right".

SPACE

Plans for UK-6

PLANS for a UK-6 satellite to follow the Science Research Council's UK-5 are being drawn up. At present, UK-5 is the last of a series of scientific satellites that the SRC is financing; the launch is planned for late this year. But, although no firm decision has yet been taken, low cost, pre-project definition for a UK-6 satellite is taking place.

Three experiments are being considered for UK-6, the chief of which is being devised by Professor Peter Fowler and his group at the University of Bristol. The plan is to investigate heavy particles in the cosmic radiation, particularly those with atomic number $Z > 70$. The design is unique and consists of a sphere 74 centimetres in diameter filled with a mixture of argon and nitrogen which scintillates when a charged particle passes through it. Within this sphere is a spherical shell of plastic 61 centimetres across in which Čerenkov light is emitted as a particle passes through. Information from both parts of the detector is necessary to pin down the charge of the particle and it is intended that both pulses of light will be detected by the same photomultiplier. A Čerenkov pulse of this kind has a spread of only about 20 nanoseconds whereas the scintillation takes about 70 nanoseconds to reach its peak and is about 300 nanoseconds long. The result is a double-peaked photomultiplier pulse. Information from the pulse can either be telemetered directly back to Earth or recorded on tape and relayed when the satellite is over Britain. The other experiments under consideration are two X-ray studies, one from the University of Leicester and the other a joint project between the Mullard Space Science Laboratory and the University of Birmingham.

The Science Research Council said this week that plans are at a very early stage and that approval for UK-6 to be built and launched has yet to be obtained.

ESRO

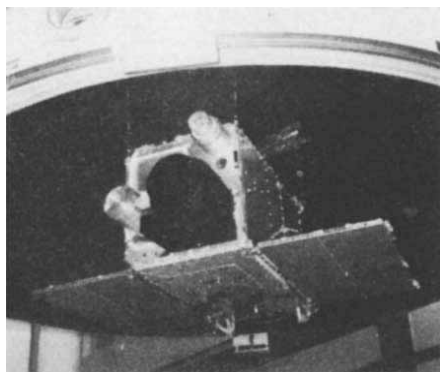
TD-1A Wakes Up

EUROPE's largest and most expensive satellite, TD-1A, has been successfully reactivated after almost four months unplanned hibernation. Launched in March last year, the satellite is now to complete the ultraviolet and gamma-ray survey of the sky that was interrupted last November when the satellite entered partial eclipse in the northern hemisphere.

Originally the systematic star survey in the ultraviolet and gamma wave-

lengths was to be completed in the six months after launch. But in May the tape recorders on this, Europe's first astronomical satellite, failed, and provision was made to put the instruments into hibernation instead of closing them down permanently in case ESRO decided to continue the programme.

The hibernation procedure carried out in November is described as the most complex operation yet attempted by ESRO's operations centre at Darmstadt. Six hundred and eleven commands had to be carried through in 50 minutes without a hitch. Last week's de-spinning and reactivating of the spacecraft was carried out in stages from Tuesday to Saturday.



—ESRO

Structural model of TD-1A

Preliminary tests reveal that none of the instruments and sub-systems has suffered degradation during the shut-down, and the second scan may last until October 1973 when the craft will again enter eclipse. A period during May is particularly important as measurements that were lost last year when the tape recorders failed need to be repeated. When the second tape recorder on TD-1A failed in May, ESRO had to gather data in real time. At first only about 30 per cent of the data were being recovered on each orbit, but by the autumn, with the help of nearly 40 additional ground tracking stations, one of which was aboard a ship in the Pacific, this was boosted to about 60 per cent. Both NASA and the French CNES provided extra ground support. ESRO says that preliminary examination of the TD-1A data shows that approximately 50 per cent of the scientific work planned for the satellite was completed by last November. Seven stations have been added to the normal ESRO tracking network including Ahmedabad (India) and Mexico City. With these in operation, the second scan should increase sky coverage to about 85 per cent. Little has so far been published of the findings from TD-1A's seven experiments. The most striking results so far produced by the £8 million satellite have come from its largest experiment, the ultraviolet sky-scan telescope.

OECD

PCBs to be Controlled

THE manufacture and sale of PCBs are to be controlled. The OECD council decided in Paris last week that member countries may not use PCBs except for certain specific jobs where their non-flammability is essential. These include dielectric fluids for transformers, heat transfer fluids (but not in food and drug processing plants), hydraulic fluids in mining equipment and in capacitors. Otherwise PCBs may not be used either industrially or commercially.

Member countries will report to council in 1974, 1975 and 1976 on progress. Countries are encouraged in the council's decision to eliminate the use of PCBs as plasticizers pesticides, vacuum pump fluids, hydraulic liquids and heat transfer fluids (in the food and pharmaceutical industries), and companies are to be requested to use replacement materials "which are less hazardous to human health and the environment than the range of PCBs now in use".

Over the next three years OECD members are to exchange information on the amounts of PCBs manufactured, imported, exported and consumed and on the replacements for PCBs proposed by manufacturers. Methods of PCB disposal will also be discussed. Safety measures for containers and for transportation, a uniform labelling system and safe disposal systems are to be created by member countries.

The council's action follows mounting evidence that polychlorinated biphenyls are both toxic and persistent. Since 1966 when traces of PCBs were found in poisoned fish and wild birds, concern about the damage they cause has grown.

In 1971, a total of 48,400 metric tons of PCBs were produced in the OECD countries and concerted action by all the organization's members should do much to reduce the problem, because as far as OECD is aware, only its members actually manufacture PCBs in the western world.

In the long run the only use of PCBs that can be permitted, the council feels, is in large scale closed circuit long-life systems, which in effect means in transformers and in large capacitors. Recovery of PCB from these systems is practicable. In all other uses recovery is impracticable and the council wants these uses banned and suitable substitutes found.

The council is also eager that information on replacement products should be collected and reviewed. It would be ironic if, when replacements were in use, PCBs proved to be the lesser of the evils.

NEW WORLD

AEC Plans Peaceful Explosion but Raises Anger

by our Washington Correspondent

The Atomic Energy Commission has announced that it will soon set off an underground nuclear explosion in a remote area of Colorado in an attempt to free natural gas trapped in a deep layer of sandstone. The blast is part of a programme to test the feasibility of using nuclear explosives to extract natural gas from deposits which cannot be worked economically by conventional means. But, in addition to several million cubic feet of slightly radioactive gas, the explosion is likely to bring to the surface a good deal of bitter controversy.

If all goes according to the Commission's plan, three nuclear explosives, each producing a yield of 30 kilotons, will be placed in a vertical line, about 450 feet apart, in the gas-bearing sandstone. They will be fired simultaneously, and the blasts are expected to produce cavities which will join to form a huge cylindrical cavern some 150 feet across and 1,300 feet high, filled with broken rock. The blasts will also smash the surrounding sandstone, releasing natural gas which will then flow into the cavern. The idea is to keep the gas sealed in the cavern for several months to allow short lived radioisotopes to decay. Some of it will then be brought to the surface, its radioactivity will be measured and it will be burned.

Called Rio Blanco, after the Colorado county in which it will take place, the test will be the third and the largest in the AEC's gas stimulation project, which is all that now remains of the Plowshare programme—a venture conducted jointly by the AEC and industry which is designed to find peaceful uses for nuclear weapons technology. The project may eventually lead to commercial production of natural gas by nuclear blasts, but at this stage it is entirely experimental.

The justification for going ahead with Rio Blanco, according to the AEC, is that all-embracing catchphrase "the energy crisis". Because natural gas contains very few pollutants, it is becoming more and more desirable for a variety of uses, but there is estimated to be only about 12 years' supply in workable deposits in the United States. In a deep layer of sandstone underlying the Rocky Mountain area, however, there is reckoned to be at least another ten years' supply but because the sandstone is relatively impermeable, just sinking a

well will not release enough gas to make the effort worth while. Hence this attempt to make the sandstone more porous by smashing it up with nuclear explosives.

The Rio Blanco shot itself will be fired off sometime after March 31, in a sparsely populated region of Colorado called the Piceance Creek Basin. Although the AEC is at pains to point

out that the test does not imply that there will definitely be further explosions in the area, if Rio Blanco is judged a complete success—the gas yield, radioactivity and structural damage caused by the seismic shock will all be taken into consideration—it would be followed by four or six more tests a couple of years later. The third and final testing stage would be a pilot programme

AUTO EXHAUSTS

Standards in Doubt

by our Washington Correspondent

A COMMITTEE of the National Academy of Sciences reported last week that automobile manufacturers should be capable of producing automobiles by 1975 which meet the stiff emission control standards specified by the Clean Air Act. But its conclusion also carried some severe qualifications, and the committee is much more equivocal about the manufacturers' ability to produce cars which meet the even stiffer requirements for 1976 models. The Clean Air Act specifies that 1975 model cars must emit 90% less carbon monoxide and hydrocarbons than 1970 models and that by 1976, emission of oxides of nitrogen should also be cut by 90% when compared with 1971 models.

The NAS committee reported that four types of engine seem likely to meet the 1975 standards — conventional engines equipped with catalytic converters, the Wankel engine equipped with an exhaust thermal reactor, the diesel engine and the new carburetted stratified charge engine which is being developed chiefly in Japan. But the committee also believes that there must be provision in the act for one change of catalytic converter in the 50,000 durability testing and that for all the engines, emissions must be averaged during the tests. If these qualifications are allowed, the committee believes that the technology is available to meet the 1975 standards.

As far as the 1976 standards are concerned, however, the committee reports that although five different systems have been tested successfully at low mileage, all have failed after a few thousand miles, and there is considerable doubt about the manufacturers' ability to mass produce engines to satisfy the demand. The most promising engine to meet the 1976 standard

seems to be the dual-carburetted stratified charge engine, which now seems to be cheaper, more dependable and more economical to run, but it is unlikely to be as readily available as conventional engines with a dual catalyst system. These conclusions have led the committee to warn that the American automobile industry seems to be putting most of its eggs into one basket by concentrating on catalytic converters, but the stratified charge system seems the best bet in the long run.

In a letter transmitting the report to Congress and to the Administrator of the Environmental Protection Agency, Dr Philip Handler, president of the National Academy of Sciences, points out that the cost of catalytic converters, combined with the extra costs associated with loss of mileage per gallon because of the emission controls, add up to a total annual expenditure for emissions control of about \$2,700 million. This cost, he suggests, should be carefully weighed against the benefits, and its effect on such factors as GNP, balance of payments and so on should be carefully studied. In particular, he suggests that attention should be paid to the possibility of enforcing the emissions standards only in those areas "where air quality is known to be adversely affected by automotive emissions", and reserving national implementation until more reliable and less expensive emission control devices are available.

Automobile manufacturers have always maintained that emission control technology will not be available in 1975 to meet the standards imposed by the Clean Air Act, and they requested one year's delay in implementation of the controls. After a lengthy public hearing, however, Mr William D. Ruckelshaus, Administrator of the Environmental Protection Agency, denied their request. But a few days before the NAS committee published its report, a federal appeals court directed him to reopen the hearings.

in which between 20 and 60 wells would be blasted out to produce sufficient gas to justify a major pipeline. Complete development of the Rio Blanco gas field would entail stimulation of up to about 300 wells.

The test will be the first to employ several explosives, and it will also use a new explosive device designed to produce a low yield of volatile radioactive isotopes, particularly tritium. Two previous explosions in the gas stimulation project, called Gasbuggy and Rulison, produced gas yields between five and ten times higher than would be produced by sinking a single borehole into the sandstone layer, but the gas was too heavily contaminated with radioactivity to be used commercially.

The chief objectives of the Rio Blanco test are first to measure the improvement in gas yield by using multiple explosives and, second, to see whether the new device can cut the radioactivity down to an acceptable level. Another part of the experiment will be to remove water and also tritium from the gas and to re-inject these products into the ground through a second borehole. If that manoeuvre is successful, the radioactivity in the gas would be approximately halved. Once brought to the surface, the gas will be burned in the atmosphere. If the water cannot be re-injected into the ground, it will simply be fed back into the gas stream and vapourized.

The project could ultimately lead to the commercial production of natural gas containing small quantities of radioactivity, and there is certain to be strong opposition from environmental groups. The AEC reckons that if nuclear stimulated gas is used for domestic purposes, a user could be exposed to less than an extra 1 per cent of natural background radiation, but there is also a chance that the explosions will cause structural damage to buildings in the area. Several organizations last week said that although they are vehemently against the project, they have so far no plans to try to stop at least the Rio Blanco test.

The most bitter opposition is likely, however, to come from the oil industry. The Rio Blanco gas deposits lie directly underneath what is believed to be the richest oil shale field in the world, and several oil companies are lining up to bid for rights to develop the field. They believe that their operations could be hurt by Rio Blanco, and that they could be completely devastated if the gas field were ever fully developed commercially. The Department of the Interior is planning to lease oil shale fields in Colorado, Utah and Wyoming to oil companies, and is at present preparing a final environmental impact statement on the proposed leasing. The impact statement should be ready in a few weeks, and leasing could take place a month after

its publication—just at the time when Rio Blanco is due to be fired.

Oil shale developers are worried that gas stimulation blasts in the Piceance Basin may fracture the oil shale strata, which would make mining more difficult, that they would collapse the mines themselves and that they would damage the retorts on the surface which are used to distil oil from the shale. Partly for these reasons, officials in the Department of the Interior and also the governor of Colorado, Mr John A. Love, have been lukewarm or even opposed to the gas stimulation plans for the area. But they have somewhat reluctantly given their consent to Rio Blanco, although their support for any further blasts in the area cannot be guaranteed. Mr Rogers Morton, Secretary of the Interior, and Dr Dixie Lee Ray, the new chairman of the Atomic Energy Commission, have jointly given assurances that the Rio Blanco test will not damage the oil shale fields.

The oil companies point out that the Piceance Basin contains an estimated 500,000 million barrels of oil, which would contribute considerably more to solving the energy crisis than the natural gas which is likely to be derived from the area. The royalties which they would pay to the state of Colorado from development of the oil shale are also likely greatly to exceed any royalties from the production of gas. Asked last week whether legal action is likely to be taken to try to prevent Rio Blanco taking place, an executive of one interested oil company said that "we will continue to oppose this project and we shall have to decide what action will be necessary".

Apart from the oil companies, some residents of Colorado are not happy about the Rio Blanco test and the developments which may follow from it. They are worried about two chief consequences—seismic damage from the blast and radiation exposure. In an environmental impact statement published in April last year, the AEC estimated that Rio Blanco would cause about \$50,000 worth of damage to buildings in the area, and the commission is recommending that work be suspended in mines nearby and that some buildings be evacuated.

A spokesman for the Colorado branch of Environmental Action pointed out last week, however, that the AEC predicted in 1969 that the Rulison blast would cause no seismic damage at all, but eventually ended up by paying out about \$100,000 in damages. "We just can't trust what the AEC tells us", he said. Moreover, if a single test is estimated to cause \$50,000 worth of damage, what will be the effects of complete development of the field with some 300 gas stimulations?

The AEC points out, however, that it

is developing a technique for setting off explosives in sequence instead of simultaneously. The idea is to wait for the seismic shocks from one explosion to dissipate before setting off the second, and so on. This scheme would mitigate the seismic effects because the individual explosions would be much smaller, but the problem is that the explosive devices must be sufficiently rigged so that the blast from one will not affect the others. The AEC was, in fact, hoping to test sequential firing in Wyoming in 1974 in a test codenamed Waggon Wheel, but the 1974 budget for Plowshare has been slashed considerably and no money has been provided for the test. The earliest possible date for Waggon Wheel is now reckoned to be 1976.

As for radiation exposure, the chief source of radioactivity from the Rio Blanco test is likely to be flaring the gas at the wellhead, the most predominant radioisotope being tritium. The AEC's environmental impact statement suggests, however, that the maximum dose received by local residents will be less than 1 per cent of the background radiation in Colorado. The AEC is also sure that since the test will take place at about 6,000 feet beneath the surface, it will not contaminate groundwaters with radioactivity.

But Dr H. Peter Metzger, a Coloradoan who has recently published a book about the AEC (*The Atomic Establishment*, Simon and Shuster, \$8.95), sums up the fears of local residents by suggesting that if the gas field is fully developed, "the chances are that sooner or later the wastes will contact an underground water supply and, through fractures, be brought to the surface and enter the biosphere". This suggestion is hotly denied by the AEC.

Perhaps the chief criticism of the gas stimulation project is simply that it is bad technology. Several of the opponents of Rio Blanco said last week that they seriously doubt whether the nuclear gas stimulation will ever be used commercially because of the possible environmental effects and also because consumers would be unwilling to use gas contaminated with radioactivity, even at the minute levels which the AEC is predicting.

Why is the AEC so keen on the gas stimulation programme, in spite of the opposition to it? One reason is that the programme is all that is left of Plowshare. Once a grandiose scheme for using nuclear explosives for all sorts of wierd and wonderful projects, such as excavating harbours and canals, Plowshare has been forced underground because of the radiological consequences of setting off nuclear blasts in the atmosphere—not to mention the test ban treaty. And Metzger also suggests that the gas stimulation project is par-

ticularly interesting to industry because of the potentially large work volume involved (Rio Blanco is being conducted in conjunction with a company called CER Geonuclear Corporation). But the project's trump card is clearly the energy crisis.

ENVIRONMENT

Nixon's Plans

by our Washington Correspondent

LAST week President Nixon sent to Congress a message outlining his Administration's plans to clean up and protect the environment. The message was accompanied by a package of bills and by some sharp criticism of Congress for foot-dragging on key environmental legislation. But soon after the message was received on Capitol Hill, Senator Edmund S. Muskie, who has long been at odds with the Administration over environmental matters, erupted with a statement of his own in which he lambasted Nixon for being long on rhetoric but short on commitment to environmental quality.

Claiming that the Administration is "well on the way to winning the war against environmental degradation—well on the way to making our peace with Nature", Nixon sent to Congress some 22 bills, 19 of which are reformulations of bills which were introduced last session, but which either languished in committees or got lost in the backlog of legislation awaiting final action when Congress adjourned for the elections.

A chief aim of the Administration's environmental plans is the development of policies for better use of the land—the proposals submitted to Congress last week include a new power plant siting law which sets out procedures for public review and comment, a measure to protect coastal wetlands and a new bill to regulate strip mining more closely. Other bills include a proposal for regulating toxic substances, a tax on industrial plants which emit sulphur oxides in regions in which the primary air quality standards are not being met, and a sheaf of legislation designed to protect wildlife.

Muskie is upset not because the Administration is proposing all these bills, but because he feels that it is not moving forcefully enough in implementing legislation which has already been passed. He is, of course, particularly irked by the fact that Nixon tried to veto the Water Pollution Control Bill last year, and when his veto was overridden, announced that he would not spend more than half of the money which it authorized. In the light of such action, Muskie says that he is "amazed that the President expects us to believe that under his leadership the Federal Government will 'play an active, positive role'".

Among the new initiatives proposed in the Administration's package of bills are the following:

- The Administration will again ask Congress to provide authority for states and local governments to use highway trust funds for the development of mass transit systems. The Highway Trust Fund consists of special highway taxes which at present can only be used for building and improving highways, but Senator Muskie has already introduced a bill aimed at changing the law so that the funds can be tapped for mass transit systems. Although the Administration has supported such moves in the past, Russell Train, chairman of the Council on Environmental Quality, said last week that President Nixon now considers it "one of the highest priorities for action this year". The bill is, of course, being opposed by the so-called highway lobby—motorist organizations and construction companies which have consistently argued that the solution to the United States' transport problems is to bury more of the country under concrete.

- New legislation has been introduced which would give the Administrator of the Environmental Protection Agency power to establish safe standards for drinking water. Water suppliers would be required to notify customers whenever the standards are not met, and although the states would retain the chief enforcement powers, the Federal Government would step in if a hazard seems imminent.

- The sulphur tax proposal is being reintroduced this year, after a thorough review within the Administration and in spite of the fact that it has little support within the House Ways and Means Committee, to which it will be referred. Late last year, it seemed likely that the proposal would be dropped because of the shortage of low-sulphur fuels, but the Administration now believes that the technology for scrubbing stack gases will be available by 1976, a year after the primary air quality standards must be met. The proposal is to levy a tax of 20 cents per pound of sulphur emitted and the tax would come into effect in 1976 only in those areas in which the primary air quality standards—which are designed to protect health—are not being met. In addition, the Administration is proposing that the tax should be imposed in 1979 in those areas in which the secondary air quality standards—which are designed to protect vegetation, property and aesthetic values—are not being met. The idea is to provide an extra incentive for companies to clean up their smoke stacks and also to force low-sulphur fuels to be allocated to regions where they are most required.

Although the message and the pack-

age of bills provides a blueprint for the Administration's environmental policies, a more important message in this area will be submitted to Congress in the next few weeks. It will be President Nixon's long-awaited message on energy policy, in which he is expected to outline plans for meeting the energy crisis. The environmental impact of such plans—for example, he is expected to recommend increased use of coal—will be watched closely, but of more far reaching import will be the Administration's revised plans to revamp the federal bureaucracy along functional lines. One of the proposals will be the creation of a Department of Natural Resources, but exactly what such a department would contain is still an open question. The environmental message made no mention of bureaucratic reorganization, and it is now expected to come with the energy message.

Short Notes

Technology Assessment Board

Now that the House of Representatives has finally got round to sorting out its committees for this session, the board of the Office of Technology Assessment has finally been appointed. It was announced recently that Mr Olin Teague has been appointed to take the place of Mr Earle Cabell, now departed from Congress, and that Mr Morris Udall of Arizona will take the place of Mike McCormack, who has relinquished his place on the board because of his commitments on the new subcommittee on energy.

HEAO

WHAT is left of the High Energy Astronomy Observatory programme, already a casualty of budget cuts (see *Nature*, 241, 83 and 306; 1973), may now be a pawn in further political moves. Originally envisaged as a very large automatic observatory, HEAO has been deferred while attempts are made to find ways in which some of the original objectives could be achieved more cheaply. The obvious way to do this would be by a continuation of the Orbiting Astronomical Observatory series, with which the Goddard Space Flight Center has now achieved a degree of success, notably with Copernicus (OAO-C). But if rumours now circulating are to be believed, the rump of the HEAO programme will be taken over by the Huntsville team who have great experience of developing large rockets (such as Saturn) but none of scientific satellites. The reason for the switch? To avoid embarrassing layoffs at Huntsville, now there are few big rockets wanted.

NEWS AND VIEWS

Does Polar Wandering Occur?

WHEN the few palaeomagnetists of the early twentieth century discovered that the directions of the ancient geomagnetic field, as derived from rocks, apparently differed from the direction of the contemporary field, two principal explanations were open to them. The first was simply that the results were not what they purported to be. To most scientists of the day, the idea that a rock could record the direction of the ambient field at the time of its formation, and that the record could maintain its stability over millions (or even thousands of millions) of years, must have seemed quite preposterous; and this no doubt accounts, in some part, for the fact that palaeomagnetism attracted very little serious attention until the late 1940s and the early 1950s. It is one of the ironies of scientific history, of course, that the first palaeomagnetists were working almost exactly at the time that Wegener was proposing continental drift. Continental drift was not readily accepted either, although for different reasons; and it is interesting that the chief conceptual objection to drift (the lack of viable mechanism) was overcome some forty years later just as palaeomagnetism was providing convincing evidence that drift had in fact occurred.

The second explanation occurring to the pioneers of palaeomagnetism was that the results reflected a true difference between the past and contemporary fields; and as the early workers had hoped that palaeomagnetism would provide information about the ancient field, this positive explanation proved the more attractive. Lack of systematic data precluded anything more than the general conclusion at this stage; but by the early 1950s it had become clear not only that the past and present fields apparently differ but that the difference has some sort of systematic relationship with time. For a brief period the more detailed results seemed to be consistent with the idea that the geomagnetic pole had moved with respect to the geographic pole. This was (geomagnetic) polar wandering in its most basic and literal sense. It is worth noting, however, that ancient geomagnetic poles were derived from palaeomagnetic data on the assumption that the field has always been dipolar. Thus an hypothesis that some of the early workers had set out to confirm or refute (the very existence in the past of a pair of geomagnetic poles) now appeared as a basic assumption, although it was not inconsistent with the data derived from rocks.

In so far as an historical thread is discernible among the rapidly changing concepts of the period, the next, practically simultaneous, step was the proposition of the first, and so far only, viable theory for the origin of the geomagnetic field—that the field results from motions in the Earth's fluid core. Because such motions must be governed in part by the effects of the Earth's rotation, one consequence of the core origin theory is that the geomagnetic axis must, on average, lie along the rotational

axis, the only unique axis in the Earth (although, in practice, the geomagnetic axis appears to oscillate about the rotational axis with a standard deviation of about 11°). Thus it became apparent that the ancient geomagnetic pole could not have wandered from the geographic pole to the extent demanded by the palaeomagnetic data. This placed palaeomagnetists in a dilemma which was soon resolved by the realization that the same effect could be achieved if the whole Earth had moved relative to the axis of rotation (now conceptually locked to the average geomagnetic axis). If the Earth as a whole slips with respect to the rotational axis (which remains fixed relative to the Sun, apart from precession and nutation), it is clear that a rock becoming magnetized will not be able to distinguish between such a slip and a wandering of the geomagnetic pole from the geographic pole. The movement of the whole Earth relative to the rotational (geomagnetic) axis is now termed true polar wandering or simply polar wandering. A variant of it, which would achieve the same palaeomagnetic effect, is a movement of the crust (or lithosphere) as a whole with respect to the rotational axis. In this case the mantle could be regarded as fixed relative to the rotational axis with the crust (lithosphere) sliding over it as a single block or shell.

But one of the consequences of polar wandering is that all rocks of the same age that give a valid palaeomagnetic result at all should lead to the same position of the ancient geomagnetic pole, irrespective of where on the Earth's surface they acquired their magnetization. It soon became clear, however, that this consequence was not confirmed by observation. Although each continent seemed to have a relatively simple polar wandering path with time, the paths were different for different continents, at least for about the past 200 million years. The only possible explanation for this, assuming the field always to have been dipolar, is that continents have not only moved relative to the poles but also relative to each other.

Continental drift during about the past 200 million years (Wegenerian drift) is now almost universally accepted. But at this point it may be asked to what extent the measured continental motion is continental drift *per se* and to what extent polar wandering is also involved—in other words, is all the measured movement of continents strictly relative movement or is there also a component of non-relative movement caused by the rotation of the whole Earth or crust (lithosphere) with respect to the rotational axis?

This is not an easy question to answer. It is not difficult to distinguish palaeomagnetically between polar wandering and continental drift if one or the other acts alone; but if drift and wandering occur together, the drift effectively obscures the wandering. Because the only available frame of reference in the Earth is the

rotational axis, combined drift and wandering is mathematically (and palaeomagnetically) indistinguishable from drift, whatever may have happened mechanically. It is for this reason that polar movements derived from palaeomagnetic data are often referred to as apparent polar wandering. Thus apparent polar wandering can represent relative motions between continental blocks (continental drift), a component of motion common to all continental blocks (polar wandering), or a combination of the two.

So how can polar wandering, if present, be detected in a continental drift situation? Duncan *et al.* (*Nature*, **239**, 82; 1972) recently attempted to do it, making use of the mantle plumes postulated by Morgan (*Nature*, **230**, 42; 1971). They assumed, first, that mantle plumes are fixed relative to the mantle, and second, that igneous activity not associated with plate boundaries is a consequence of the surface effects of plumes, bands of igneous activity being caused by the passage of lithospheric plates over the mantle-fixed plumes. There are dangers here, of course. For one thing, plumes may not exist, and even if they do the two assumptions may not be valid (as Duncan and his colleagues freely admit). But the attractive conceptual point is that the fixing of the plumes to the mantle gives an absolute frame of reference which, in theory, enables polar wandering to be separated mathematically from continental drift. If polar wandering alone takes place by a rotation of the whole Earth with respect to the rotational axis, a mantle-fixed plume will also remain fixed to a continent or lithospheric plate. If, however, continental drift (or lithospheric plate motion) occurs, a plume trace will be produced as the continent moves over the plume. The continental drift proper may then be derived from the plume trace and subtracted from the apparent polar wandering (continental drift plus polar wandering) to give the true polar wandering.

By analysing the igneous activity (assumed plume traces) in the Central Volcanic province of Europe and the Thulean province of Iceland, the Faeroes and the British Isles, Duncan *et al.* concluded that plume and palaeomagnetic data are only consistent if true polar wandering of about 23° has taken place since the lower Tertiary. But on page 523 of this issue of *Nature*, McElhinny concludes independently that during the past 50 million years the lithosphere as a whole has not slipped significantly with respect to the rotational axis. The two conclusions are clearly incompatible. At this stage the most reasonable inference must be that plumes, if they exist, cannot be fixed to the mantle, although it may also be overenthusiastic to regard every volcano as a hot spot.

P. J. S.

Stratospheric Molecules

THE development of supersonic airliners has renewed interest in the chemical composition of the stratosphere. In particular there has been a lively debate about whether the passage of such an aircraft through the stratosphere will initiate changes in the chemical balance. The projected cruising altitudes of Concorde and its Russian

rival are much lower than the ozone layer, and are therefore much less liable to deplete it than the supersonic airliner proposed in the United States in 1971. Another source of changes in the composition of the stratosphere has been the programme of nuclear tests, which may possibly have depleted the ozone shield by 5 to 7 per cent by causing the injection of nitrogen oxides. The result of this interest has been an expansion of studies of the upper atmosphere, particularly by spectroscopic methods, which are less susceptible to local contamination by the observation system. By combining data collected from various observation platforms, such as aircraft, balloons and satellites, it is gradually becoming possible to chart the global distribution and vertical distribution of the important chemical constituents of the atmosphere.

Recent work by Harries, reported on page 515 of this issue of *Nature*, predicts some useful data on the worldwide distribution of some of the important chemicals found in the stratosphere. Measurements of the submillimetre far-infrared emission spectra of the stratosphere were made from Concorde 002 during the tour of the Far East in June 1972. The analysis of the spectra has provided concentration data for water vapour, ozone, nitric acid, nitrous oxide, nitrogen dioxide and sulphur dioxide. Many of these compounds can be produced during fuel combustion, and their formation at much lower altitudes was mentioned in *Nature* recently (**241**, 341; 1973). Harries's preliminary study indicates that there are no significant variations of the chemical composition with latitude, but the results suggest that the vertical distribution, particularly for nitric acid, may well be non-linear. The concentrations of water and ozone measured in this way are of the order of 4 parts per million above the tropopause, which was generally at an altitude of between 40,000 and 50,000 foot. Nitrous oxide, an important link in the nitrogen cycle, has a concentration about 10 per cent of that of water in this region. Nitric acid, nitrogen dioxide and sulphur dioxide have much lower concentrations which are about 0.1 per cent of that of water.

The results that have been obtained so far, averaged over altitude, tropopause height, and location, do not reflect any of the known seasonal trends. It is known that the ozone concentration does vary with time and location, and it would be interesting if the present experiments could be extended to more southerly latitudes than 40° S to see whether there is any polar increase. Some of the other species of interest, such as nitric acid, are thought to be associated with ozone, as suggested by Murcray *et al.* (*J. Opt. Soc. Amer.*, **59**, 1131; 1967), and so may well exhibit similar fluctuations. Some transient species such as NH and NH₂ have probably been identified in the stratosphere by their electronic spectra, but their concentrations are unknown. Measurement of their pure rotation spectra would enable good estimates to be made of their abundance, for their dipole moments are either measured (in the case of NH), or estimable (in the case of NH₂).

Further experiments in this series should provide data which may answer some of the questions posed above, and may allow vertical concentration profiles to be derived for various localities. The information gained from this and similar experiments at varying altitudes should lead to the construction of an improved model for the chemical processes in this region.

From a Correspondent.

Synthetic Hepatitis B Vaccine ?

CONSIDERABLE efforts are currently being directed towards passive and active immunization against hepatitis B infection. There is some evidence that passive immunization, using gamma globulin prepared from plasma containing high titre antibody to hepatitis B antigen, offers significant, though temporary, protection against infection. Trials with heated whole serum containing hepatitis B antigen have been carried out in New York (see *Nature*, **233**, 92 ; 1971) and more recently in Paris. Heat-inactivated whole serum, however, can hardly be regarded as a satisfactory or indeed as an acceptable "vaccine".

An immunochemical study of the purified antigen is essential for the understanding of this unique antigen. Analogous to the tobacco mosaic virus coat protein decapeptide the primary sequence of the haptenic peptide of hepatitis B antigen may provide a basis for a development of a synthetic peptide which, when coupled to human albumin, could serve as a suitable immunogen. In last Wednesday's issue of *Nature New Biology* (**241**, 240 ; 1973) K. R. Rao and G. N. Vyas describe the preparation of active subunits of hepatitis B antigen by sonication and chromatography. This may provide a lead for the preparation of a "synthetic" material containing the immunogenic determinant. Isolated viral coat protein challenges the body's immune mechanism in the same way as the whole infectious agent. It is widely believed now that hepatitis B antigen (Australia antigen) represents incomplete viral particles in the form of aggregates of protein subunits or excess viral coat protein.

Once detailed data are available on the protein, peptide and amino-acid composition of hepatitis B antigen, it should be possible to define by animal immunization the moiety responsible for the antigenic activity. Infectivity studies can be carried out in appropriately selected subhuman primates such as rhesus monkeys and, in due course, possibly also in culture preparations of liver.

There are reports in the literature to support the feasibility of such an approach. For example, in 1966 J. M. Stewart and colleagues (*Biochemistry*, **5**, 3396 ; 1966) defined the antigenic moiety of TMV protein decapeptide.

R. Arnon and her colleagues (*Proc. US Nat. Acad. Sci.*, **68**, 1450 ; 1971) showed that it was possible to use a synthetic macromolecule for eliciting antibodies reacting exclusively with a specific region of a native egg-white lysozyme. This was achieved by synthesizing a particular segment of the enzyme from its amino-acid components, attaching the peptide to a synthetic polypeptide carrier and using the conjugate for immunization. The resulting antibodies reacted with native lysozyme in a unique, conformation-dependent antigenic determinant. Of course, in the case of lysozyme both the amino-acid sequence

and the three-dimensional structure are known, and the synthesized peptide was designed on the basis of previous information concerning its contribution to the antigenic specificity of the molecule.

A similar approach might be attempted for type B hepatitis, depending largely on the success in elucidating the structure of hepatitis B antigen. There is little doubt that polypeptides and other moieties such as specific lipoproteins can be attached to a macromolecular carrier (M. Sela, *Adv. Immunol.*, **5**, 29 ; 1966) for subsequent immunization. Speculative? Perhaps, but an exciting challenge nonetheless.

From our Medical Virology
Correspondent.

Caps and Patches on Lymphocytes

OPTIMISM is a sterling attribute for the biological scientist who would otherwise readily become bewildered by the complexities of the materials under study. Lymphocytes are thought of and manipulated by many contemporary cellular immunologists who tend to think of them as units of function in immune responses. But it has been apparent for some time that the interaction between a lymphocyte and the antigens to which it responds is susceptible of investigation at the boundary of the cell with its environment—the plasma membrane.

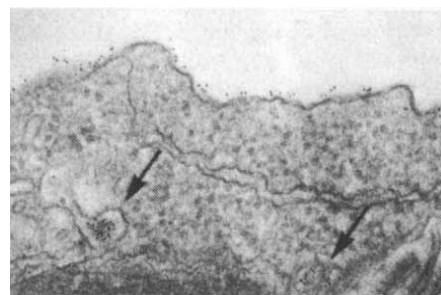
It has been shown (Taylor *et al.*, *Nature New Biology*, **233**, 225 ; 1971) that attachment of cross-linking antibodies to the immunoglobulins which are found on the surfaces of some lymphocytes can cause migration of the bound molecules to one pole of the cell and the formation there of a "cap". This process of capping *in vitro* requires metabolic activity by the cell and could not be induced by using monovalent antisera. In an article in next week's *Nature New Biology* (February 28) de Petris and Raff carry these investigations further.

In the earlier report Taylor *et al.* used fluorescent anti-immunoglobulins to cause capping and as a visual indicator of the process. In the new studies de Petris and Raff used ferritin-bound antibodies followed by electron microscopic examination by which the ferritin molecules can be visualized and their locations used as a manifestation of the process of capping (see figure). Their new conclusions bear out the results of the earlier studies and make two important additional observations. First, they find that when divalent cross-linking ferritin-bound antibodies are used to cause cap formation there is a prior formation on the cell surface

of patches, which represent dense aggregates of antibody bound to the immunoglobulin target molecules. These patches are not observed when monovalent antisera are used and they do not therefore represent the distribution of immunoglobulins in the unperturbed membrane but are a consequence of cross-linking. Interestingly, and unlike capping, patch formation can occur in the presence of metabolic inhibitors such as sodium azide and at low temperatures.

Second, de Petris and Raff find that pinocytosis can occur on a small scale and after some time without cap formation when a monovalent antiserum has been used. They therefore conclude that cap formation and pinocytosis are independent phenomena.

These are interesting studies but it should be emphasized that an *in vivo* equivalent to cap or patch formation is not yet known. Neither is it yet obvious what role either process has in relation to the performance of the affected cell in an immune response. On the optimistic hypothesis that because these processes exist they must have biological significance, the further development of this work should be of great interest.



Arrows point to small, ferritin-containing, pinocytotic vesicles in section of lymphocyte.

TUMOUR BIOLOGY

Chemical Transformants

from our Cell Biology Correspondent

THE properties of chick embryo fibroblasts infected and transformed by certain temperature sensitive mutants of Rous sarcoma viruses have established that the maintenance of the transformed cell phenotype depends upon the continuous expression of one or more viral functions. Similar experiments with a solitary temperature sensitive mutant of polyoma virus lead to the same conclusion. Both the RNA and the DNA tumour viruses have small genomes and it seems unlikely, to say the least, that each and every change in the phenotype of the cell transformed by one of these viruses results directly from the action of a distinct viral protein. The viruses simply do not have enough genetic information to be so profligate. It follows therefore that the limited number of transforming proteins specified by the tumour viruses must induce either pleiotropic effects or they must interact with various cellular molecules and indirectly bring about transformation.

At present the nature of the transforming viral proteins is not known, neither is anything known about the putative cellular molecules which may be targets for the viral proteins. But just as it is possible to inactivate by mutation the viral genes involved in transformation, it ought to be possible to mutate the cellular genes specifying the target molecules, and Di Mayorca *et al.* (*Proc. US Nat. Acad. Sci.*, **70**, 46; 1973) may have done this; certainly the data they present can be so interpreted.

Di Mayorca *et al.* exposed BHK₂₁ clone 13 cells to dimethylnitrosoamine or nitrosomethylurea and then plated the cells in liquid media to measure their plating efficiency and in soft agar to measure their ability to grow in suspension into colonies. These BHK cells do not grow in agar suspension unless they have been transformed and this technique of suspension plating has been widely used as a method of selecting and assaying for transformation of BHK cells. Using these procedures Di Mayorca *et al.* detected transformants at a frequency of 6×10^{-6} in populations of BHK cells exposed to appropriate doses of either dimethylnitrosoamine or nitrosomethylurea. That transformants arise after exposure to these chemical carcinogens/mutagens is not in itself particularly surprising, but the phenotype of the transformed cells is remarkable. Apparently all the transformants isolated after the cells have been exposed to the carcinogens as well as a single clone of spontaneously transformed BHK cells prove to be temperature sensitive for the two markers of the transformed

state that were assayed—the ability to grow in agar suspension and the morphology of the colonies. In all cases these transformants failed to grow in agar at 32° C and in liquid media grew to form typical monolayers, but at 38.5° C they behaved as transformed cells growing to form colonies in agar and to form multilayered foci in liquid medium.

These results are apparently fully reproducible and the properties of these chemical transformants are in marked contrast to the properties of BHK cells transformed by polyoma virus which exhibit the transformed phenotype at 32° C and 38.5° C. These temperature sensitive chemical transformants also differ from cells transformed by temperature sensitive tumour viruses, the latter having a transformed phenotype at lower temperatures and an untransformed phenotype at higher temperatures.

The obvious interpretation of these data which Di Mayorca *et al.* offer is that the cells they have isolated carry a temperature sensitive lesion in a cell gene which specifies a product required for the maintenance of the normal or untransformed phenotype. They suggest that at 32° C the putative cellular gene product is functional whereas at 38.5° C it is inactivated because of a temperature sensitive mutation and as a result the cells acquire the transformed phenotype as their temperature is raised. Clearly if such a cellular gene product, that is required to maintain a cell in the untransformed state, exists it must be inactivated when a cell is transformed by a tumour virus; it must presumably be inactivated, for example, when

BHK cells are transformed by polyoma virus and this putative cellular protein may be a target of the viral transforming gene proteins.

No doubt such speculations are not to everybody's taste, but surely few would deny that the cells which Di Mayorca and his colleagues have isolated have remarkable properties.

TEMPERATE VIRUSES

Infection in Humans

from our Medical Virology Correspondent

SUBACUTE sclerosing panencephalitis (SSPE), a fatal disease of children and young adults, is a chronic progressive infection of the central nervous system by a measles-like virus (M. Katz *et al.*, *Nature*, **222**, 888; 1969; and V. Ter Meulen *et al.*, *Curr. Topics Microbiol. Immunol.*, **57**, 1; 1972). The evolution of the disease can be divided into clinical stages characterized by mental and behavioural changes, disorders of the locomotor system and loss of cerebral-cortex function. The SSPE agent has many of the biological properties of measles virus, but unlike conventional strains of measles it remains intimately bound to cells and it has a different pattern of replication and intracellular behaviour.

The chronic nature of this viral infection, the possibility that the formation of immune complexes may be important in the aetiology of this disease and perhaps in causing some of its damage to nervous tissue (A. D. Dayan and M. I. Stokes, *Brit. Med. J.*, **2**, 374; 1972) suggest that SSPE may be a model of temperate viral infections in humans, possibly an important group

Transformation by Herpesvirus

SOME herpesviruses of mammals are known to possess an oncogenic potential, and Duff and Rapp (*Nature New Biology*, **233**, 48; 1971) believe that herpes simplex virus, a human virus, can transform hamster cells. Whether herpes simplex virus or any of the other herpesviruses which are indigenous to man have any role in the development of cancer in human beings is, however, another and a vexed question. Obviously human herpesviruses, which can be shown to transform human cells in culture, must be seriously considered as potential human carcinogens and for this reason numerous groups are carrying out the appropriate experiments. Darai and Munk in Heidelberg, for example, have been investigating the infection of human embryonic lung cells with *Herpesvirus hominis* type 2, and they report in next Wednesday's *Nature New Biology* (February 28) that when cultured in appropriate

conditions the infected cells have some of the properties characteristic of transformed cells.

The complete replication of *Herpesvirus hominis* in cultures of human lung cells results in cell lysis, and to prevent this Darai and Munk raised the temperature of the culture 1 hour after infection from 37° to 42° C. Cells which survived infection for eight days at 42° C were then returned to 37° C and repeatedly subcultured. Several cell lines were established and these cells were tested in a variety of ways.

The cells all proved to contain a *Herpesvirus hominis* antigen in the cytoplasm; to tend to form syncytia as a result of cell fusion apparently promoted by a viral-coded cell surface like a protein; to resist superinfection and to survive longer in culture than control uninfected cells. These properties suggest that the cells may have been transformed.

of disorders in man including such divergent diseases as viral hepatitis (A. J. Zuckerman, *Immunopathology* VI, edit. by P. A. Meischer, Schwabe, Basel, 1971, p. 436) and multiple sclerosis (F. Wolfgram *et al.*, *Multiple Sclerosis, Immunology, Virology and Ultrastructure*, Academic Press, 1972). This is now the subject of intensive investigation in many laboratories.

Traditionally the tissue damage caused by viral infections has been explained by the ability of viruses to multiply in cells, thereby leading to cell injury or destruction. It is now becoming clear, however, that other mechanisms may be operating and that lesions may be caused by the immune response of the host to viral antigens and that the immune system itself may be disturbed by some viruses. Thus, certain viruses depress the humoral and cellular immune responses of the host, whereas other viruses may enhance the antibody response. Several viral antigens persist in the circulation and combine with specific antibody resulting in antigen-antibody complexes. These complexes may lodge in various sites, and in other instances combination with complement releases substances which cause tissue damage. Immunological injury mediated by antibodies may also result when new antigens produced by viruses on infected cell surfaces interact with specific antiviral antibody and complement (Memorandum: Virus-associated Immunopathology, *Bull. Wld Hlth Org.*, **47**, 25; 1972).

This concept of virus-associated immunopathology has recently been extended to temperate viral infections and it is possible that the ability to produce infectious immune complexes may be important in establishing chronic infections such as SSPE and hepatitis B in man and lymphocytic choriomeningitis, aleutian mink disease and murine sarcoma and leukaemia in animals. Chronicity may be attributable to differences in the affinity of the antibodies produced, a factor which is partly under genetic control, perhaps on mechanisms linked to histocompatibility genes (W. F. Bodmer, *Nature*, **237**, 139; 1972). It is difficult, however, to explain the relative rarity of SSPE, if it is caused by measles virus, in the face of the ubiquitous distribution of measles. What is needed is an animal model, but experimental inoculations of animals with different strains of SSPE agent have generally yielded divergent results. P. Albrecht and colleagues (*J. Infect. Dis.*, **126**, 154; 1972) have reported recently that a hamster-brain-adapted strain of measles virus produced encephalitis in rhesus monkeys with morphological findings similar to those described in SSPE and quite unlike acute measles encephalitis in man. The principal property respon-

sible for this potential of measles virus seems to be an aberrant intracellular replicative cycle of the virus which affects the maturation process at the cell membrane. Further understanding of temperate viral infections in humans thus seems likely in the near future.

PHOTOPHOSPHORYLATION

Without Membranes

from our Photosynthesis Correspondent
In 1954 it was discovered that isolated chloroplasts could utilize light energy to make adenosine triphosphate (D. Arnon *et al.*, *Nature*, **174**, 394). This finding had important implications because it meant that chloroplasts could produce ATP as well as NADPH₂ for driving CO₂ fixation. Photophosphorylation is now a well documented phenomenon which can readily be detected with lamellae fragments obtained from chloroplasts which have been broken osmotically. It has even been shown to occur in small subchloroplast vesicles obtained either by sonication (R. E. McCarty, *J. Biol. Chem.*, **244**, 4292; 1969) or by treatment of inner lamellae with digitonin (N. Nelson *et al.*, *ibid.*, **245**, 143; 1970). The common feature of these phosphorylating systems was that they were dependent on the presence of an intact membrane and also they required the addition of electron carriers or acceptors. It now seems possible, however, that photophosphorylation can occur in the absence of both intact membranes and artificially supplied electron carriers.

McPhee and Brody (*Proc. US Nat. Acad. Sci.*, **70**, 50; 1973) report the

striking observation that an acetone extract obtained from chloroplasts when layered on an aqueous surface will exhibit light-induced phosphorylation. The extract was obtained from particles derived by sonicating spinach chloroplasts, and was used to produce a monolayer at an air-water interface by compression to a surface tension of about 20 dynes cm⁻¹. Electron microscopy indicated the films to be partly composed of small structured particles of about 200 Å diameter at a concentration of one particle per 2 × 10⁸ Å² of compressed surface. On illumination McPhee and Brody detected ATP production with quantum yields between 0.1 and 1. The ability of the monolayer to catalyse photophosphorylation was dependent on maintaining a compression of at least 10 dynes cm⁻¹ and could be doubled by adding phenazine methosulphate.

The artificial system was also found to induce additional esterification of inorganic phosphate not directly correlated with ATP formation. To explain their results McPhee and Brody argue that the phosphorylation observed may take place by a two-step reaction, one involving transphosphorylation between two ADP molecules to yield AMP and ATP followed by esterification of AMP to ADP.

Although McPhee and Brody have not used their data to discuss the chemical (E. C. Slayter, *Eur. J. Biochem.*, **1**, 31; 1967) or chemiosmotic (P. Mitchell, *Biol. Rev.*, **41**, 445; 1966) hypotheses their observations will undoubtedly be used by the "anti-chemiosmotic school" in attempts to dislodge Mitchell's elegant hypothesis.

Poly(A) Tract and Messenger RNA Ageing

THE function of the tracts of adenylic acid residues that are known to occur at the 3' ends of many messenger RNA molecules remains a mystery, but experiments reported by Sheiness and Darnell in *Nature New Biology* next Wednesday (February 28) indicate that as messengers age in the cytoplasm of HeLa cells the length of the poly(A) tracts decreases; this may be a clue to their function.

Sheiness and Darnell analysed the poly(A) liberated by ribonuclease from populations of messenger RNAs in cytoplasmic extracts of HeLa cells fed pulses of ³H-adenosine and then actinomycin D. They conclude from such experiments, first, that the tracts of poly(A) in cytoplasmic messengers shorten as a result of metabolic ageing and, second, that this shortening occurs in the cytoplasm after the messengers have been transported from the nucleus. Furthermore, this time-dependent shortening of the poly(A)

tracts occurs when protein synthesis is blocked. It seems plausible to suggest therefore that the lifetime of a messenger may be determined by the length of the 3' poly(A) tract and the rate at which the messenger loses adenylic acid residues. In other words, the function of poly(A) tracts of messengers may be to programme the lifetime of the molecules.

Also in *Nature New Biology* next week Gross *et al.* report the isolation from the polysomes of sea urchin embryos of a "9S" RNA fraction which stimulates a cell-free system from Krebs II mouse ascites cells to incorporate amino-acid. Fingerprint analysis of the product of *in vitro* translation indicates that it includes histones, and by comparing the stimulation of incorporation of tyrosine and tryptophan by 9S RNA Gross *et al.* conclude that their 9S RNA seems to be relatively free of contamination by messengers for proteins other than histones.

NUCLEIC ACIDS

Conformation Contorted

from our Molecular Biology Correspondent

GONE are the days when DNA was seen as nothing more than a repository of information, which did nothing but sit in the chromosome waiting to be transcribed. It is still by no means clear that the canonical structural forms, other than the B structure, which is presumed to be that of native DNA in aqueous media, have any functional significance, although the rather ill-founded notion is gaining ground that DNA in the chromosome is partly in the C form. What does seem clear is that the potential for conformational change in response to environmental disturbances is built into the molecule, and with it a rich harvest of speculation, ripe for the gathering.

The dearth of hard information, especially in regard to the state of DNA when associated with basic molecules, is such that a study by Suwalsky and Traub (*Biopolymers*, **11**, 2223; 1972) on the X-ray diffraction of such complexes comes as an agreeable change. They have examined nucleoprotamine from sperm heads, and also complexes with polylysine and polyarginine as a function of humidity. The principal upshot is that, at least at high humidity (and therefore by implication in solution), the DNA is in all cases in the B form. The structures have not been explicitly solved, and probably complicated by intermolecular interaction modes. It seems clear, however, that in both nucleoprotamine and the polylysine complex the polypeptide chain is strung along the narrow groove of the double helix. In spite of the high arginine content of protamines, the polyarginine seems to bind in a different manner. At low humidity, the DNA in the polylysine complex goes into the C form, but in the presence of polyarginine this transition does not take place. The results should perhaps help to discourage some of the more uninhibited interpretations of the very striking optical properties of such complexes, which tend to invoke unusual DNA conformations.

Interesting optical effects also occur in complexes of DNA with large amounts of histones, which have been systematically explored by Fasman and his associates and by others. More data on the circular dichroism of complexes with F1 histone have now been collected by Šponar and Frič (*ibid.*, 2317). The circular dichroism in these systems is changed in shape and grossly intensified, and Šponar and Frič find that whatever interaction causes this phenomenon is a function of base composition, the largest effects occurring with DNA of high GC content. The

curves rather resemble those of Lerman's ψ -form, which is characteristic of DNA in solutions containing salts and neutral non-interacting polymers. Interpretation in such situations tends to run far ahead of the data. That it is all fraught with meaning may, however, be apparent from the results of Adler, Langan and Fasman (*Arch. Biochem.*, **153**, 769; 1972), who have found a remarkable effect on the circular dichroism of the complexes with F1 histone of phosphorylation. This occurs enzymatically at two serines, and the result is that the effect of the histone on the circular dichroism is substantially eliminated. Phosphorylation of either serine gives about half the effect. Merely introducing a few new negative charges, as by chemical maleylation, has no such influence on the optical activity. Sedimentation experiments indicate that the phosphorylation does not noticeably affect the affinity of the DNA for the histone, which is very basic. It therefore seems as though some structural feature of the interaction of F1 is altered by phosphorylation, which occurs physiologically at the two sites.

It may come as no surprise that the miasma of allostery is now beginning to engulf DNA. Pohl *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 3805; 1972) have come up with an interesting system, in which a structural change in DNA is indeed triggered by a ligand, and because of the high conformational cooperativity associated with long DNA helices the effect is rather dramatic. Like other DNAs, the alternating polymer, poly (dG.dC), evidently undergoes some form of structural change in solutions of high ionic strength,

which is characterized by an inversion of Cotton effects in the ultraviolet circular dichroism. This is a slow first-order intramolecular process. Now the dye, ethidium bromide, binds to DNA by intercalation between the base pairs, in this particular DNA to the extent of one dye to two base pairs at saturation. What Pohl *et al.* have now found is that at high salt concentration the binding to a polynucleotide of high molecular weight becomes acutely cooperative, and goes from zero to full saturation within an almost imperceptibly narrow range of dye concentration. This is most felicitously explained in terms of what is indeed a kind of allosteric effect, whereby the low-salt form of the DNA has a very high, and the high-salt form a very low, affinity for the dye. At a critical dye concentration the conformation is therefore sucked abruptly into the low-salt form.

Circular dichroism of the dye complex at high and low ionic strength confirms that the conformations are similar. The kinetics, monitored by the fluorescence change of the ethidium on binding, are consistent with the demands of such a scheme. The value of the "allosteric constant", the equilibrium constant relating the low and high-salt forms in the absence of dye, which best fits the data, agrees with the value obtained by earlier work on the salt-induced transition of the polymer. Whether such finely poised transitions of DNA occur in life is, of course, at this stage anybody's guess.

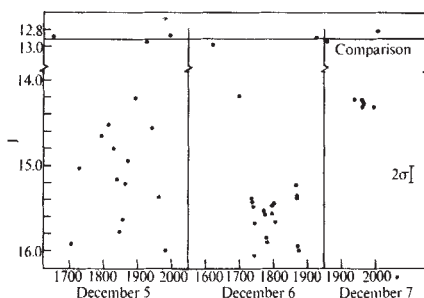
A quite different type of conformational problem is the prediction of RNA structures when there is only partial internal complementarity. Im-

Rapid and Strong Variations in BL Lac

In next Monday's *Nature Physical Science* (February 26) Weistrop reports observations of the unusual object BL Lac carried out at the Florence and George S. Wise Observatory in Israel. BL Lac is intriguing because it exhibits many of the properties of QSOs but has

no lines in its visible spectrum. It has been speculated that this object, and two or three similar objects, might be blueshifted QSOs.

Weistrop's observations were made on three nights in December 1972. They show evidence of extremely strong short-term variability in the source (see diagram) during periods much less than 1 h on December 5, 6 and 7. Previously, other observers have noted changes in the brightness of BL Lac of a few tenths of a magnitude in one night and by as much as 1.3 mag within 20 h. The present data indicate changes of 1.5 mag within 1 h, and there is a hint of repeated patterns occurring over 85 min. On at least two occasions, says Weistrop, variations of 0.5 mag occurred within a few minutes. All this evidence points to the occurrence of extremely violent processes in BL Lac, making the object even more of a prime target for further observations.



Variability of BL Lac (lower part) compared with a standard reference star observed by the same telescope on the same nights.

portant steps in this direction are revealed in two articles, which treat of the stability of short helices, joined by loops of unpaired bases. In a molecule such as $A_nC_mU_n$, the existence of a flexible link of C residues will favour the pairing of the A with the U, when m is not too large or too small. Uhlenbeck *et al.* (*J. Mol. Biol.*, **73**, 483; 1973) have made a series of such molecules with $n=6$ and m varying between 4 and 8. When the loop is only four or five residues long, it is evidently strained, and the probability of A-U pairing is less than theoretically expected; indeed circular dichroism gives evidence of an unusual geometry for the single-stranded cytosine residues when $m=5$. The most favourable situation occurs at $m=6$. Gralla and Crothers (*ibid.*, 497), working with the oligomer series $A_nGC_nU_n$, arrive at similar conclusions. If $n=4$, the loop is too tight and, in spite of the entropy loss, intermolecular interaction is preferred, because to make an easier intramolecular loop, the G-C pair would have to be prised open. In the range n from 4 to 6, the intramolecular fold thus becomes more stable with increasing loop size. The thermodynamics and statistical mechanics of these systems are treated in both articles in a manner to which one cannot do justice without a fuller account. Some interesting implications regarding tRNA conformation can be drawn.

STEROIDS

Testicular Oestrogens

from a Correspondent

It has long been recognized that males and females secrete the same type of steroid hormones and the difference between the sexes is quantitative, the ovary producing predominantly oestrogens and the testis producing predominantly androgens, in particular testosterone. Many of the hormones, in addition to direct secretion from the gland, may arise by the peripheral conversion of precursors.

A large amount of circumstantial evidence suggests that in man the testis secretes oestrogens: oestrogenic material can be found in extracts of testes and testicular tissue incubated *in vitro* can convert suitable precursors to oestrogens. Application of constant infusion techniques and measurement of the steroids both in blood and urine, however, has produced conflicting evidence; some results suggest that about half of the circulating oestrogens in the male are secreted by the testis, the remainder arising from circulating precursors, whereas other experiments suggest that there is no direct secretion from the testis. There is now direct evidence to support the former view.

In one study (Longcope *et al.*, *Steroids*, **20**, 439; 1972) young males, who were undergoing renal vein catheterization as part of an investigation of essential hypertension, had samples of blood removed by catheterization from the inferior vena cava below the entrance of the spermatic vein and a second sample was taken from the spermatic vein itself. The concentrations of testosterone, oestradiol and in some cases oestrone were higher in the spermatic vein samples than in the samples from the inferior vena cava. These findings confirm the report of Kelch *et al.* (*J. Clin. Invest.*, **51**, 824; 1972) of a positive gradient for oestradiol across the testis in anaesthetized humans. The testis, like the ovary, seems to secrete more of the 17 β -hydroxysteroid (oestradiol) than the 17-oxosteroid (oestrone) in contrast to the adrenal cortex which secretes predominantly the 17-oxosteroid. The cellular origin of testicular oestrogen and particularly its function remain to be determined.

VISION

A New Slant

from our Experimental Psychology Correspondent

THE discoveries by Hubel and Wiesel of neurones in the visual system, each specifically sensitive to a line segment or edge in a particular orientation, have been regarded as of great significance and importance. Yet in spite of widespread interest, and in spite of the fact that these discoveries are now fourteen years old, there is still little clue as to the role of these cells in perception.

Hirsch and Spinelli (*Science*, **168**, 869; 1970) and Blakemore and Cooper (*Nature*, **228**, 477; 1970) seemed to hold out some hope for insights into function by showing that in cats reared in environments where they saw only horizontal or vertical lines, neurones in the visual cortex responded only to stimuli at or near the orientation to which they had been exposed. And indeed this effect of early environment on responsiveness of cortical neurones seems relatively clear. But now Hirsch (*Exper. Brain Res.*, **15**, 405; 1972) has reported on the behavioural experiments which form the complement of this research, and these indicate that the effect of the physiological changes on perception still defies adequate explanation. Hirsch's experiments were on some of the cats in his orientation-specific rearing series which had had electrophysiological recordings made from their visual cortices. When they had recovered from this procedure Hirsch ran them in some discrimination tasks.

The experiments are a little unsatisfactory in that only six of the cats reared with horizontal and vertical visual environments were tested, and in some tests only two of these animals took part. Thus what differences there were between the groups and between the experimental animals and normal controls cannot be regarded as very reliable. It is the finding that cats could perform visual tasks involving shape and orientation discrimination at all with such substantial reduction of their orientation-sensitive neuronal apparatus that is striking.

These cats were raised with all their visual experience in the light confined

Differences between Red Sea Brine Pools

OCEANOGRAPHER DEEP, which is the most northerly of the brine pools in the Red Sea, was discovered in 1967 by echo sounding from the research vessel Oceanographer. In next Monday's *Nature Physical Science* (February 26) Tooms and his colleagues of Imperial College, London, report the analysis of a sediment core recovered during a cruise of the tug Nereus in 1971. The bottom of Oceanographer Deep is about 570 m below the seafloor on either side, and is outside the median valley of the Red Sea. It is about 2 to 3 nautical miles long and 0.6 nautical miles wide about 200 m from the bottom.

Analysis of the core and the water in the core liner above the sediment revealed that although the water is a concentrated brine—the chlorinity of 16 per cent is quite similar to that of Atlantis II Deep—the amounts of trace metals such as iron and copper are very low by comparison with brines from other deeps in the Red Sea. For

example the 56° C brine from Atlantis II Deep contains 0.08 g kg⁻¹ iron by comparison with 0.2 mg kg⁻¹ for Oceanographer Deep and 0.02 mg kg⁻¹ for normal seawater.

Tooms *et al.* say that the core itself has concentrations of metallic elements comparable with those reported for anoxic Norwegian fjords. The smell of H₂S associated with the core and the fact that there was considerable shrinkage during storage indicate that the original environment of the core was strongly reducing.

One of the possible explanations of the brine in Oceanographer Deep suggested by Tooms *et al.* is that it arises from the dissolution of evaporite deposits in surrounding seawater rather than from tectonic activity. They go on to suggest that similar brines might be present in depressions in the seabed of, for example, the Mediterranean Sea where evaporite deposits are known to occur.

by wearing translucent goggles, so that one eye saw only a field of horizontal stripes and the other only a field of vertical stripes. In the electrophysiological experiments the orientation-sensitive neurones that were found responded to the orientation of rearing for the eye being tested and these cells could typically not be affected by stimuli shown to the other eye. Nevertheless, behaviourally Hirsch found that in these animals discrimination of shapes and orientations trained entirely with one eye could be performed (though somewhat less well) when tested on the other.

The findings of Hirsch with discrimination of orientations around vertical and around horizontal are perhaps the clearest. Here cats tested with the eye reared on vertical stripes performed better than with the eye reared with horizontal stripes in the discrimination of orientations around vertical. Similarly when using the eyes reared with horizontal stripes and tested around horizontal they were better than when using those reared with vertical. And cats reared normally did a little better still.

The important finding, however, was that differences between the groups were rather small and cats did manage to perform discriminations between those orientations for which they evidently lacked cortical neurones. Further electrophysiological study of these same cats following the behavioural experiments confirmed that the deficit of neurones sensitive to orientations other than those with which they were raised still existed.

One might have supposed that orientation-sensitive neurones would at least be used in making discriminations between lines of different orientation, but perhaps even this must be rethought. One cannot, on the basis of these experiments, immediately dismiss the idea of neurones in the visual cortex as feature detectors, but once again the brain seems to be more subtle and versatile than one would have given it credit for.

POPULATION ECOLOGY

Fighting a Forest Pest

from our Soviet Correspondent

A NEW mathematical theory of insect population dynamics may facilitate the preservation of forests from xylophagous insects without the excessive use of insecticides, which may themselves be harmful to standing timber. This approach, developed by A. S. Isaev, of the Sukachev Forestry Institute, and R. G. Khlebopros, of the Siberian Branch of the Soviet Academy of Sciences (*Dokl. Akad. Nauk SSSR*, **208**, 225; 1973), is based on the concept of a

"critical" population density at which ecological balance breaks down and the establishment of the "multiplication factor"—the ratio of insect density in the $(n+1)$ th generation to that in the n th.

Isaev and Khlebopros estimated the multiplication factor as a function of population density itself, the quantity and type of food, various external factors independent of the population density and the lag in the natural regulatory processes of forest ecology. They found that, for a constant type of food, a stability level should be established, around which population density should fluctuate in a manner dependent on the availability of that food. But as certain types of insect, notably the horned beetle *Monochamus urusovi*, increase in population density, regulation of the population by overconsumption of their food does not occur; above a certain "critical" density, the feeding habits of such insects become modified to include a great range of plants. When this "critical" density is exceeded, the possibility of a second stability level of population in equilibrium with food supplies does, of course, exist; in practice, however, this will not occur until there has been a massive disruption of the ecology and widespread devastation of the forest, such as *Monochamus urusovi* has produced in recent years in the fir forests of Central Siberia.

To remedy this situation, in localities where the local population density of

insects has increased above the critical level, Isaev and Khlebopros suggest the use not of insecticides but of insect repellents which, without harming the trees, would disperse the insects into surrounding areas and thus reduce the population density below the critical level. This would create a situation in which the natural regulating factors of forest ecology would gradually reduce the density of the population.

OCEANOGRAPHY

Polygons and Waves

from a Correspondent

LONGUET-HIGGINS, of the University of Cambridge, has discovered a new and remarkably simple approximation for the profile of the steepest possible gravity waves which can occur in deep water (*Proc. Roy. Soc. Lond.*, **A331**, 445; 1973). The usual conditions for a gravity wave are that the fluid be inviscid, incompressible and irrotational, that surface particles move with the surface, and that the pressure be constant over the surface. Expressed mathematically, these conditions give rise to non-linear equations which, strictly speaking, have never been completely solved.

The simplest approximation is to assume that the slope of the surface is everywhere very small (say less than 1 in 10). One may then ignore the non-linearities, and obtain the well-known

More about Aerodynamics of Buildings

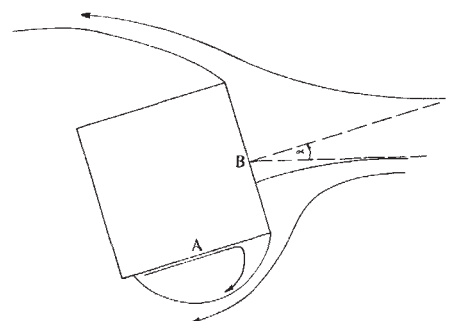
ONE of the more important things that have to be taken into account when designing a building is how the structure will behave in a wind. To this end many people have in the past examined what happens when cylinders of square cross-section, typifying many buildings or their component parts, are exposed to rapid air flow in a wind tunnel.

In *Nature Physical Science* next Monday (February 26) Mulhearn describes such an experiment which he carried out in the 4 foot by 5.5 foot closed-circuit wind tunnel in the Engineering Laboratories at the University of Cambridge. He used a cylinder with 6-inch sides which was exposed to a wind of velocity 70 foot s^{-1} (about 50 mile h^{-1}).

Earlier investigators, such as Vickery, established that the resistance of a square cylinder to wind decreases when the flow becomes turbulent. Vickery contended that turbulence promotes reattachment of the flow, which originally separated from the leading face of the cylinder (see diagram), at a position closer to the leading edge than is the case for non-turbulent flow. Bearman and Kavur, however, found that no reattachment occurred in cases where

the angle of incidence α was small.

Mulhearn's observations neatly suggest a compromise, in that he finds no reattachment at small angles of incidence but that the phenomenon does occur at larger values. He has confirmed the earlier finding that reattachment occurs sooner when the flow is turbulent, and he says that this implies that, even when reattachment does not occur, the shear layers emanating from the leading corner are closer to face A.



What is still not clear, however, is why resistance should be decreased as the separated shear layer comes nearer to the surface.

linear wave solutions with sinusoidal profiles and dispersive properties. Going one step further, the non-linear terms may be partially restored by substituting the first approximation to obtain the "Stokes wave" (Lamb, *Hydrodynamics*, art. 250, Cambridge University Press, 1932), which has flatter troughs, and crests of greater curvature, than the sinusoid. This tendency increases as the steepness is allowed to increase until ultimately, Stokes showed, a limiting form for the progressive wave should be obtained, which has a sharp symmetrical wedge-shaped crest enclosing a perfect angle of 120° (maximum slope 30° from the horizontal). Unfortunately, the method of trying to approach the 120° crest by a series of approximations from a sinusoid fails to converge.

Stokes worked in the second half of the nineteenth century; since then a succession of mathematicians have attempted by various means to formulate the full profile of the 120° wave. Basically they all give slightly different results (for the ratio of height to wavelength, for example), and nearly all involve numerical computations which are strenuous even by modern standards. The standing wave presents similar problems and, by contrast, is limited by a 90° crest. It is interesting to observe that although the limiting standing wave has a peak vertical acceleration of $-g$, so that particles at the crest are momentarily "weightless", the corresponding acceleration for the limiting progressive wave nowhere exceeds $-\frac{1}{2}g$.

Longuet-Higgins has derived his formula by the elegant trick of conformally transforming a regular polygon. A hexagon transforms into a wave-like profile with perfect 120° crests; a square yields 90° crests, corresponding to the limiting standing wave. All the hydrodynamical conditions are satisfied except that of constant pressure, but the actual pressure distribution is shown to be within 7 per cent of uniformity. And the profile is so close to the results of laborious numerical approaches as to be nearly indistinguishable on an ordinary graphical plot. Its greatest virtue is its simplicity. With suitable scaling the profile may be written

$$y = \ln \sec x$$

with trough at $x=0$ and crests at $x = \pm\pi/6$ (progressive wave) or $\pm\pi/4$ (standing wave). Simple formulations also exist for the fields of velocity and acceleration. These properties will appeal to marine engineers, who are notoriously sceptical of abstruse mathematical formulae but very concerned with the properties of the steepest waves which are precisely those responsible for the wrecked oil rig or the capsized trawler.

MATERIALS

Anomalies in Perspex

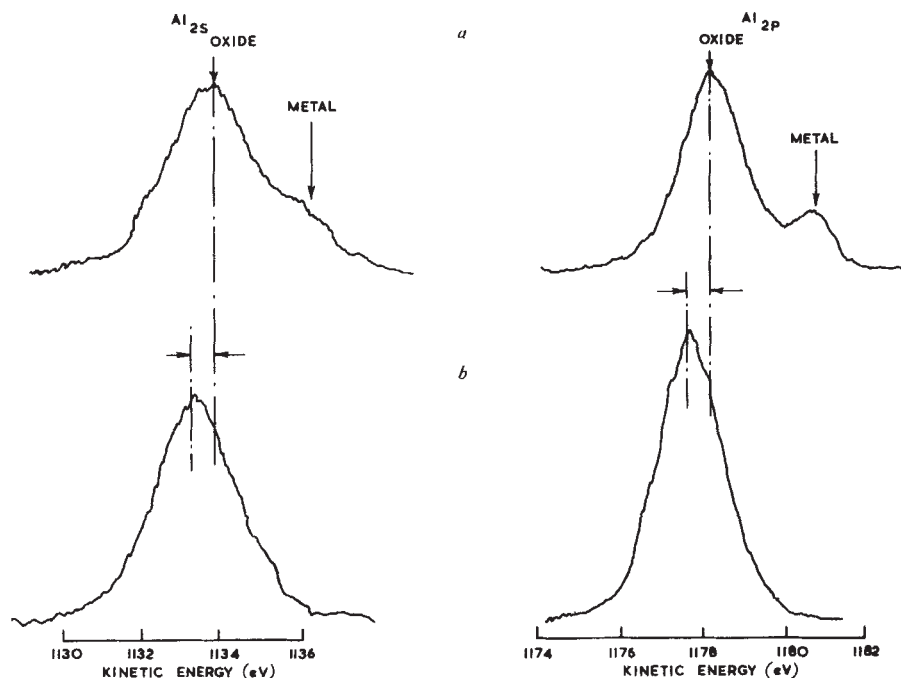
IN the latest of a series of reports about undergraduate projects which have produced worthwhile results, Puttick, of the University of Surrey, describes some curious features of Perspex in *Journal of Physics E: Scientific Instruments* (6, 116; 1973). Much of the experimental work was carried out by Jewers, then a third-year undergraduate at the University of Surrey, under supervision.

The experiment involved a study of how Perspex indents and fractures when a steel ball bearing (3.2 mm) is driven into the surface at speeds of, typically, between 0.017 and 1.7 mm s⁻¹. The work revealed some interesting features

of the way in which cracks develop under these circumstances, but it also brought to light a strange phenomenon that was observed to particular advantage when specimens were unloaded before fracture could occur.

An example is shown in the figure; a spherical region of anomalous refractive index is generated beneath the indentation, and this region defines the inner boundary of the crack, which Puttick likens to a sycamore seed. By applying elementary optics to what amounts to a thick lens embedded in a medium, it turns out that the difference in refractive index is $+1.7 \times 10^{-2}$ and that the change in density in the spherical region, $\delta\rho/\rho$, is 2.9×10^{-2} . By comparison, assuming that all the material that was in the crater (apart

ESCA Probes the Oxide Films on Aluminium



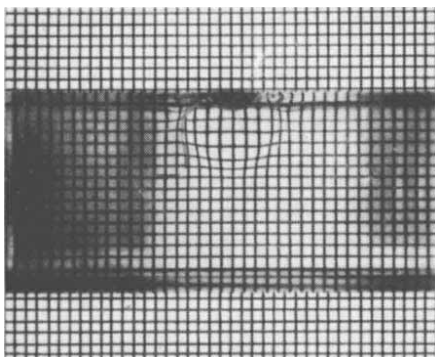
a, Hot-rolled aluminium sheet; b, similar sheet heated in an oxygen-rich flame.

ACCORDING to the literature on the subject, the most recent of which is dated 1954, the thickness of the oxide film ($\gamma\text{Al}_2\text{O}_3$) on aluminium manufactured at temperatures above 400°C can be as large as 2,000 Å. Why then does aluminium produced in this way have a shiny appearance? In next Monday's *Nature Physical Science* (February 26) Tripathi and Clark describe measurements made by ESCA (electron spectroscopy for chemical analysis) which show that the thickness of the film is in fact about 15 Å.

They have used an electron spectrometer to demonstrate that certain photoelectrons released from the metal (as opposed to the oxide) during irradiation with $\text{MgK}\alpha_{1,2}$ X-radiation could not escape if the thickness of the oxide layer

were any more than 15 ± 10 Å. The situation is illustrated in the diagram; the smaller peaks in a are caused by photoelectrons from the metal and the larger ones by electrons from the oxide layer. The spectra in b are for a piece of aluminium sheet that had been exposed to an atmosphere rich in oxygen for 3 min at 600°C . Further oxidation apparently proceeds smoothly.

Clearly the oxide film has increased in thickness to the extent that the photoelectrons from the metal can no longer escape. And Tripathi and Clark also point out that there is a shift in the position of the peak in the two situations, suggesting that when the oxide film is thick there is no longer any electrical contact with the spectrometer so the sample charges up.



The anomaly below the indentation.
The crack can just be seen to its left.
(From *J. Phys. D.*, 6, 117; 1972.)

from that piled up at the surface) has been transferred into the spherical volume, the calculated value of $\delta\rho/\rho$ is 3.2×10^{-2} . Puttick says, however, that the agreement between these values may be misleading, for experiments on hydrostatic compression of Perspex suggest that, at the maximum pressure of 3 kbar exerted in the indentation experiments, $\delta\rho/\rho$ should only be 3×10^{-4} . (But there is another caveat here, for the Perspex beneath the indenter has also undergone plastic flow).

QSOs

Redshifts and Distances

by our Cosmology Correspondent

Bahcall and Hills have investigated the magnitude-redshift relation for the QSOs which are most luminous optically in the redshift range from 0.2 to beyond 2.0. They have found that the distribution is entirely consistent with QSOs being at the cosmological distances indicated by a Doppler interpretation of their redshifts, participating in the overall expansion of the universe (*Astrophys. J.*, 179, 699; 1973).

Efforts to resolve the nature of QSOs remain, of course, a continuing astronomical saga. With distance, velocity, absolute magnitude and mass all unknown, the problem of providing a fit between theory and observation is not difficult—but then the validity of any particular fit to the real universe is highly questionable. In some way, it is necessary to restrict the sample of QSOs being examined to, say, all those of a certain mass, at a certain distance, or of a certain brightness. Taking a leaf out of the textbook studies of clusters of galaxies, Bahcall and Hills have made the not unreasonable assumption that for any redshift z the brightest QSO that can be seen on Earth will have an intrinsic brightness close to some limiting upper value, and that it will thus provide a “standard candle”. If the redshift provides a measure of distance, the apparent brightness of the appropriate standard candle will be less for larger z ; more

specifically, the exact relation between redshift and apparent magnitude will depend intimately on the properties of the Universe as a whole.

The data used in the latest analysis come from the catalogue of DeVeny *et al.* (*Publ. Astron. Soc. Pacific*, 83, 611; 1971). Because it is important to use bins which contain the same number of QSOs, in order to have a sampling which is unbiased with respect to redshift, Bahcall and Hills have not divided the redshift range into equal increments Δz , but have taken bins of different Δz which each contain fifteen QSOs. There were 105 QSOs in the sample, filling seven bins and providing seven brightest QSOs as standard candles. These are, in order of increasing z and decreasing apparent magnitude, 3C 273, 3C 232, PKS 1354+19, PKS 1252+11, PKS 1127-14, 3C 298 and TON 1530.

Bahcall and Hills discuss likely sampling effects, such as the probability of their sample including a violently variable QSO which happened to be flaring when observed; in spite of these complications, they are able after careful statistical analysis to fit the seven QSOs to a straight line of slope 5 in the $\log z$ /apparent magnitude diagram. Two very large redshift QSOs which

cannot be included in the statistical analysis because there are not enough QSOs known with $z > 2.2$ to complete a fifteen member bin also lie close to this straight line (they are PHL 957, $z = 2.69$, and 4C 05.34, $z = 2.88$; if large redshift does indicate large distance, these must be equivalent to brightest member QSOs in order to be visible).

This evidence suggests that QSO redshifts are cosmological in origin and that there is no strong evolution of the luminosity of brightest member QSOs with cosmological epoch. Because of the wide range of redshifts covered in the analysis, this is a very powerful result. It remains possible—indeed probable—that in any group of QSOs the brightest member will not always be the same object; what the data reveal is that the physical properties of the Universe which define the brightness limits on the whole class of objects known as QSOs have remained roughly constant as the Universe itself has evolved.

The analysis used by Bahcall and Hills is not limited to QSOs, however, and provides a tool which can be applied to the investigation of other objects which have a broad luminosity function and for which redshift measurements have been made.

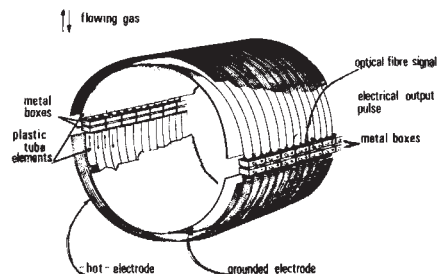
Plastic Tubes as Particle Detectors

In next Monday's *Nature Physical Science* (February 26) Conversi, the founding father of the flash tube particle detector, a device beloved of cosmic-ray physicists, describes how these detectors can be made in a flexible form from plastic materials rather than glass. As is the case for the more conventional tubes, a voltage pulse is applied across a layer of the flexible detectors a microsecond or so after the registration of a charged particle by means of a logic system; those tubes through which the particle passed will then flash along their whole length. The tubes have to be protected from extraneous light, except at one end where recording can take place either by means of a flexible optical fibre or by a probe within each tube which can pick up a small voltage pulse.

Conversi describes a particular way in which the new detector could be used with the electron-positron storage ring at Frascati (see diagram). Conversi envisages that the 2 m “experimental” straight section of the storage ring would be surrounded by two layers of tubes (only one of which is shown in the diagram). With plastic tubes of diameter 1.6 cm, 500 tube elements would be needed to cover the whole section. The vacuum chamber of the storage ring could well be the inner grounded electrode and the high volt-

age (“hot”) electrode would probably be a gauze to minimize Coulomb scattering of the charged particles.

One of the disadvantages of flash tubes as compared with, say, spark chambers is that several layers of parallel tubes will only locate the particle trajectory in two dimensions. If it is required to reconstruct particle interactions in three dimensions, then extra layers with the axes of the tube elements pointing in different directions—for example, parallel to the length of the vacuum chamber of the storage ring in Conversi's example—would have to be added.



In his experiments on the new device Conversi used a high voltage pulse about $5 \mu\text{s}$ long with a rise time of about 50 ns. The peak field was 8 kV cm^{-1} , and a mixture of neon (30 per cent) and helium (70 per cent) was pumped through the system at a rate of $1 \text{ cm}^3 \text{ s}^{-1}$.

Machines and the Theory of Intelligence

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This article is based on a series of special lectures delivered at University College, London, in November 1972.

THE birth of the subject generally referred to as "artificial intelligence" has been dated¹ from Turing's paper² *Intelligent Machinery* written in 1947. After twenty-five years of fitful growth it is becoming evident that the new subject is here to stay.

The scientific goal of research work in artificial intelligence is the development of a systematic theory of intelligent processes, wherever they may be found; thus the term "artificial intelligence" is not an entirely happy one. The bias towards artefacts is reminiscent of aerodynamics, which most people associate with aeroplanes rather than with birds (yet fruitful ornithological application has been achieved³). Here I shall review briefly some of the experimental knowledge systems which have been developed, and indicate how pieces of theory abstracted from these might fit together.

Some Performance Systems

Game playing was an early domain of interest, and Shannon⁴, Turing⁵, and Newell, Shaw and Simon⁶ contributed classic analyses of how machines might be programmed to play chess. The first significant performance system was Samuel's program⁷ for checkers, which eventually learned to play at the level of a good county player, far higher than that of Samuel himself. This last circumstance played a valuable part in discrediting the cruder manifestations of the doctrine that "you only get out what you put in".

The fundamental mechanism underlying all this work has been a cycle of processes: lookahead, evaluation and minimaxing. These derive ultimately from a method used to establish a "foregone conclusion theorem" for such games (two person, zero sum, perfect information, no chance moves) which states that the outcome value can be computed on the assumption that both players follow a (computable) best strategy. For a trivial game, such as that schematized in Fig. 1a, the computation can actually be performed: all terminal board positions are assigned values by the rules of the game, and these are "backed up" by the minimax assumption that White will always choose the immediately accessible position which has the maximum value and that Black will select the one with the minimum value. Clearly the procedure not only demonstrates a theorem but also defines a strategy.

But what is to be done when, as in any serious game, it is not practicable to look ahead to the end? Turing and Shannon independently suggested looking ahead as far as practicable, to what may be termed the "lookahead horizon", assigning some approximate values to the positions on the horizon by an evaluation function, and backing these up by the same minimax rule. The corresponding strategy says "choose that immediate successor which has the highest backed-up value".

This rule has been proved empirically in numerous game-playing programs, but in spite of its intuitive appeal it has

never been formally justified. The question is posed diagrammatically in Fig. 1b.

Search procedures form part of the armoury of the operations-research man and the computer professional. Stemming from such work as Samuel's, people concerned with game playing and problem solving have implemented mechanisms for guiding the search, first, by forming sub-problems⁸ or, second, by making heuristic estimates of distance-to-goal⁹. Various theorems have established conditions under which such techniques can be used without sacrificing the certainty of termination or the optimality of the solution found^{10,12,13}.

The use of an "evaluation function" to guide the search is a way of smuggling human *ad hoc* knowledge of a problem in through the back door. There is no cause to disdain such a route; it is after all one of the principal channels through which natural intelligences improve their understanding of the world. At the same time automatic methods have been developed for improving the reliability with which problem states are evaluated¹¹.

Samuel's early work on game learning⁷ indicated that seemingly pedestrian mechanisms for the storage and recall

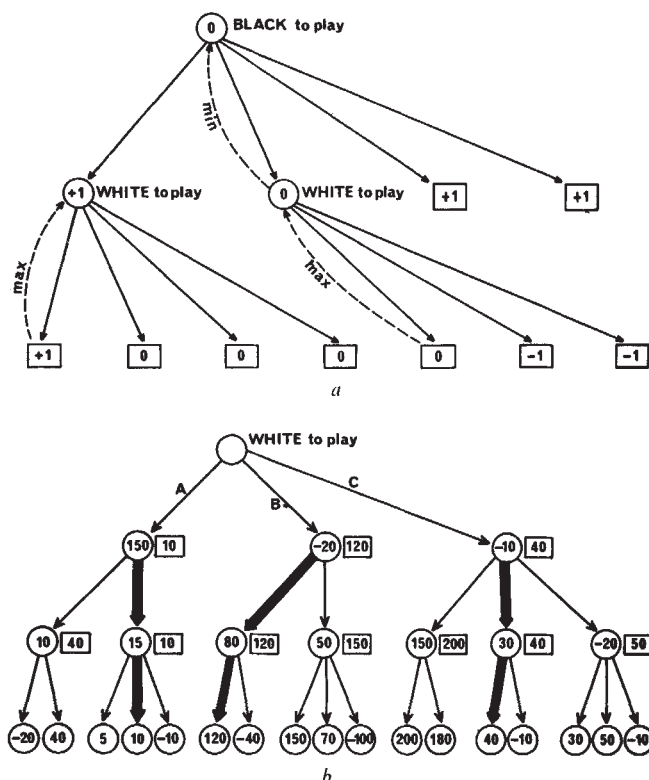


Fig. 1 a, The root of this two-level lookahead tree acquires a value by alternate application of the "max" and "min" functions. If alternation is extended backwards from all terminal positions of the game tree, the initial position of the entire game will ultimately be assigned a value. Terminal positions are shown as boxes; b, lookahead tree in which the nodes are marked with "face values". Boxed figures are values backed up from the lookahead horizon. If move-selection were decided by face values, then move A would be chosen, but if backed-up values then move B. What is the rationale for B?

of previously computed results can have powerful effects on performance. Recently the combination of rote learning schemes with heuristic search has been shown to have applications to plan formation in robots^{13,14}. To exploit the full power of this combination, whether in game playing, in robotics or in other applications, one would like the rote dictionary to contain generalized descriptions or "concepts" (for example, of classes of game-positions "essentially similar" from a strategic point of view) to be looked up by processes of recognition, rather than by point-by-point matching. Such a dictionary is to be used in the style "If the situation is of type A, then perform action x , if of type B, then action y " and so on. One is then in effect processing a "decision table" which is formally equivalent to a computer program. There is thus a direct link between work on the automatic synthesis of strategies in game playing and robotics, and work directed towards automatic program-writing in general.

Recognition usually involves the matching of descriptions synthesized from sensory input with stored "canonical" descriptions of named objects, board positions, scenes, situations and so on. Choice of representation is crucial. At one extreme, predicate calculus¹⁵ has the merit of generality, and the demerit of intractability for updating and matching descriptions of objects, positions, scenes or situations; at the other extreme lie simple "state vector" representations, which fall down through awkwardness for handling complex inter-relationships. Somewhere in the middle lies the use of directed labelled graphs ("relational structures", "semantic nets") in which nodes stand for elements and arcs for relations. Impressive use of these structures has been made in a study of concept formation in the context of machine vision¹⁶.

Language interpretation has been the graveyard of many well-financed projects for "machine translation". The trouble proved to be the assumption that it is not necessary for the machine to "understand" the domain of discourse. One of the first demonstrations of the power of the semantic approach in this area was Bobrow's "STUDENT" program¹⁷ for answering school algebra problems posed in English. A program by Woods, Kaplan and Nash-Webber¹⁸ for the interrogation in English of a data base with a fixed format has been used by NASA scientists to answer questions about Moon rocks. An essay by Winograd¹⁹ on computer handling of English language dialogue, again making intensive use of an internal model of the dialogue's subject matter, has left no doubt that machine translation can only be solved by knowledge-based systems. The knowledge base required to render arbitrary texts non-ambiguous is now recognized to be bounded only by the knowledge possessed by their authors. Winograd compares the following two sentences:

The city councilmen refused to give the women a permit for a demonstration because they feared violence.

The city councilmen refused to give the women a permit for a demonstration because they advocated revolution.

The decision to refer "they" to "councilmen" in the first case and to "women" in the second implies a network of knowledge reaching into almost every corner of social and political life.

Mass spectrogram analysis was proposed by Lederberg as a suitable task for machine intelligence methods. The heuristic DENDRAL²⁰ program developed by him and Feigenbaum now outperforms post-doctoral chemists in the identification of certain classes of organic compounds. The program is a rich quarrying ground for fundamental mechanisms of intelligence, including the systematic conjecture of hypotheses, heuristic search, rote learning, and deductive and inductive reasoning. I shall refer back to this work later in connexion with the use made by intelligent systems of stored knowledge.

Of all the knowledge systems which have been attempted, robotics is perhaps the most simple in appearance. In reality, however, it is the most complex. The chess amateur can appreciate that Grandmaster chess has depth and subtlety. But there is no such thing as a human amateur at tasks of

navigation and "hand-eye" assembly. Every man is a Grandmaster at these tasks, having spent most of his waking life in unwitting but continual practice. Not having been informed that he is a Grandmaster, and having long since stored most of his skill at a subliminal level, he thinks that what seems subjectively simple is objectively so. Experience of research in robotics is a swift and certain cure. Something of the depth of analysis which is required can be gleaned from the discussion by McCarthy and Hayes²¹ of the properties which should be possessed by a calculus of situations, actions and causal laws.

The crux of any such calculus is how to represent in a formal language what the robot knows about its world. McCarthy and Hayes distinguish "epistemologically adequate" and "heuristically adequate" representations. (In an earlier generation Ryle²² contrasted "knowing that" and "knowing how".) "The epistemological part is the representation of the world in such a form that the solution of problems follows from the facts expressed in the representation. The heuristic part is the mechanism that, on the basis of the information, solves the problem and decides what to do."

I shall consider now what is probably the simplest world to be seriously discussed, that of Popplestone's "blind hand" problem (internal report, Department of Machine Intelligence, Edinburgh), with the object of indicating that there is more to robot reasoning than meets the eye, and expanding a little the epistemological-heuristic distinction.

A blind, insentient, robot shares with one or more "things" a world consisting of only two places "here" and "there", and has available to it the actions "pickup", "letgo" and "go". "Pickup" is non-deterministic and causes (if the hand is empty when the action is applied) a "thing" selected at random from the place where the robot is, to acquire the property "held". An initial situation called "now" is defined, in which it is asserted that every thing at "here" (and there is at least one such) has the property "red". A goal situation is defined as one in which at least one red thing is at "there".

Invariant Facts and Laws

The kinds of facts which the robot needs to know include that the robot and anything held by it must be in the same place, and that something cannot be in both places at once. Using a prescription of Green²³, a formalization of this apparently trivial problem in first order logic might start along the following lines. (The variables t , p and s are to be interpreted as standing for objects, places and situations respectively.)

for all t, p, s : held(thing(t), s) and at(thing(t), p, s) implies at(robot, p, s),

for all t, p, s : held(thing(t), s) and at(robot, p, s) implies at(thing(t), p, s),

for all p, s : at(robot, p, s) implies at(thing(taken(s)), p, s),

for all t, s : at(t , here, s) implies not at(t , there, s).

The conjunction of these statements describes some of the physics of this world. The last statement, for example, asserts that an object cannot be both at "here" and at "there" in one and the same situation.

The initial situation, "now", is described in like manner:

for all t : at(t , here, now) implies red(t), at(thing(a), here, now).

The latter statement merely asserts that at least one thing (represented by the constant a) is at "here" in situation "now". The function "thing" is a convenience for distinguishing other objects from the robot, whom we may wish to exclude from some otherwise universal statements—like one implying that the robot is "held", for instance.

How can the machine be enabled to reason about the chains of possible consequences derivable from "now" and so to construct an action chain leading to a goal situation? The goal may be defined, using Green's "answer" predicate²⁴, as:

for all t, s : at(t , there, s) and red(t) implies answer(s).

But how do we handle the actions? The contrast between epistemological and heuristic criteria becomes very sharp at this point. Consider two approaches.

One can go the whole way and stick to formal logic, defining the transition laws of our world under the various actions. For example, the first of the following three "letgo" axioms translates freely: "in the situation produced by doing a 'letgo', nothing is held".

for all t, s : not held (thing(t), do(letgo, s)),

for all t, p, s : $at(t, p, s)$ implies $at(t, p, do(letgo, s))$,

for all t, p, s : not at(t, p, s) implies not at($t, p, \text{do}(\text{letgo}, s)$),

and similarly for the other actions.

Now the problem of plan construction is reduced to one of logical deduction, in fact deduction of the statement "answer(do(go(there), do(pickup, do(go(here), do(letgo, now))))". This says, in English, that "the goal situation is the one resulting from doing a "go there" in the situation resulting from doing a "pickup" in the situation resulting from doing a "go here" in the situation resulting from doing a "letgo" in the situation "now" ", and it is clear how this can be re-interpreted as an algorithm.

This deduction can in principle be mechanized, but there are two severe snags. First, the need to incorporate "frame axioms"^{24,25} (which spell out all the facts which remain unchanged after the performance of given actions, as in the last logic statement above) escalates for nontrivial problems and renders the automatic deduction process intractable even in the present toy problem. Second, the logic representation is not heuristically adequate.

On the other hand, one can go to the other extreme, and express the whole problem as a computer simulation couched in a suitable programming language, matching situations with data structures and actions with procedures. But this approach encounters difficulties with the epistemological criterion, for the structure of the problem world can be readily complicated so that it can no longer easily be described by the use of simple representations of the "state vector" type. Various attacks are being made on the representation problem in an attempt to make the best of both worlds, the epistemological and the heuristic. Some good early suggestions were made by Popplestone, using essentially the same blind hand problem, and were reviewed in *Nature*²⁷ two years ago. Since then powerful new programming aids, such as the PLANNER²⁸, QA4 (ref. 29) and CONNIVER³⁰ languages have come into play. In addition particular mention should be made of the Stanford Research Institute's study of autonomous plan formation^{14,15}, in which many of the matters discussed above have been under experimental investigation.

The key ideas on which much work centres is that plan construction should be conceived as a search through a space of states of knowledge to generate a path connecting the initial knowledge state to one which satisfies the goal definition. Everything turns on finding ways of representing knowledge states so that the transformation of one into another can be neatly computed from the definition of the corresponding action ("What will I know about the state of affairs after doing A?").

Experimental Robotics

The STRIPS system^{14,15} at Stanford Research Institute combines reasoning in first-order predicate calculus with heuristic search. In the situation depicted in Fig. 2 the robot must devise a plan for pushing objects around so that one of the boxes end up in room R1, subject to the constraint that at no time must the wedge be in the same room as a box. If the plan goes wrong, the system must be capable of recovering from error state and, if possible, "mending" the failed plan appropriately. Facilities are incorporated whereby successful

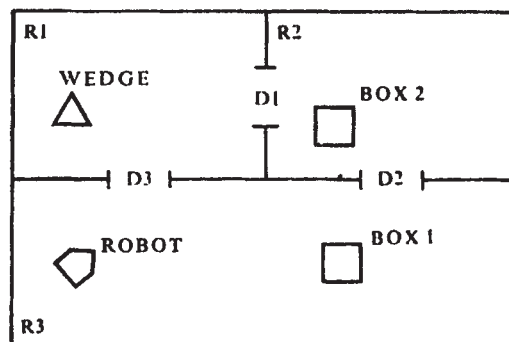


Fig. 2 Robot environment for a constraint problem. (Reproduced from ref. 31.)

plans are automatically “remembered” and their elements recombined for use in appropriate future situations¹⁴.

Following simultaneous development of the idea of optical ranging in Japan³², Britain (R. J. Popplestone, personal communication) and America³³, Stanford University's robot project uses a laser optical ranging system for mapping the three-dimensional surfaces of "seen" objects. Another branch of the same project is currently able to assemble an automobile water pump comprising two pieces, a gasket and six screws (J. Feldman, personal communication). This is done blind, using mechanical feedback.

At Edinburgh automatic assembly is also under study. Programs exist for packing simple objects onto a confined surface, identifying a limited set of objects by visual appearance, and solving problems of stacking rings on pegs (D. M., A. P. Ambler, H. G. Barrow, R. M. Burstall, R. J. Popplestone, and K. J. Turner, paper to be presented at a Conference on Industrial Robot Technology at the University of Nottingham next month).

In industrial laboratories, notably in America (for example, the Charles Stark Draper Laboratory of MIT) and Japan³⁴, automatic assembly studies are multiplying.

Idea of a Theory

I have already mentioned the abstracting of pieces of theory from performance systems such as those listed above. What is meant by "theory" in this context? I have just considered a fragment of simple robot world theory, and one can, of course, speak of a piece of chess end-game theory (for example, that expressed by Tan's program³⁵ for the two-Kings-and-one-Pawn end-game) or of the theory of mass spectrometry embedded in the heuristic DENDRAL program. One can even, legitimately, speak of Winograd's program as constituting a linguistic theory, or at least as containing or implying one. But these theories are descriptive of specific domains, not of intelligence itself.

It would be naive to pretend that the search for a meta-theory is something new, or even that it is anything but old philosophy in new dress. An early name suggested for what is now "artificial intelligence" was "epistemological engineering" (P. M. Woodward, personal communication). The new epistemology, however, has a trick which the old philosophers lacked, namely to express any given theory (of knowledge, reasoning, abstraction, learning and the like) in a sufficiently formal style to program and test it on the machine.

Hence there is no longer a meaningful distinction to be drawn between a theory of some given intelligent function, and an algorithm for carrying it out (which could in turn be converted into a program for some particular machine) together with any useful theorems for describing the algorithm's action. Algorithms, then, are theories, and this has been true for a long time. But there have been no reasonable mechanisms available for handling them. Mathematics, on the other hand, has had the necessary mechanisms for manipulating the formalisms which it uses for describing physical systems. Hence closed-form mathematics has been the "typical" embodiment

of theory in the physical sciences. By contrast, the "typical" embodiment of theory in cognitive engineering is algorithmic.

What Use is Knowledge?

The value of stored knowledge to a problem-solving program again divides into epistemological and heuristic parts. In the first place sufficient knowledge must be present for solutions to be in principle deducible. But that is only the start. Heuristically, the value of knowledge is that it offers ways of avoiding, or greatly reducing, processes of search. The natural enemy of the worker in the field of artificial intelligence is the "combinatorial explosion", and almost his entire craft is concerned with ways of combatting it. The following three examples illustrate the use of stored knowledge to damp off combinatorial explosions.

First, Tables 1 and 2 show the number of combinatorially possible ways in picture-processing of labelling various patterns of intersecting lines, contrasted with the number that are physically possible on the assumption that they arise in retinal projections of three-dimensional scenes composed of plane polyhedral bodies, such as that shown in Fig. 3a. The computer program achieves this order of reduction by the use of an appropriate theory. Here I shall review briefly a subset of the theory, adequate for interpreting line drawings of plane-surfaced polyhedra, with trihedral vertices only and without shadows. In this way the flavour can be imparted of the kind of reasoning involved in more complex cases.

Each line in such a drawing can be assigned to one or another of various possible causes: it corresponds to a convex edge, a concave edge, or to an edge formed by two surfaces, only one of which is visible. A corresponding label can be attached to each line, as has been done in Fig. 3b using Huffman's conventions³⁶. The remarkable fact emerges from Huffman's analysis that only a few of the combinatorially possible ways of labelling such drawings correspond to physically possible structures in the outside world: only twelve distinct configurations of lines around vertices are possible. A computer program can use the theoretical constraints to process the picture, by searching through the space of possible labellings for those which are legal (that is, do not entail that any line should receive two different labels) under the constraints.

Second, Table 3 contrasts the number of topologically possible molecular graphs corresponding to given empirical

Table 2 Comparison of Number of Combinatorially Possible Labellings with the Number that are Physically Possible

	Approximate number of combinatorially possible labellings	Approximate number of physically possible labellings.
	2,500	80
	125,000	70
	125,000	500
	125,000	500
	6×10^6	10
	6×10^6	300
	6×10^6	100
	6×10^6	100
	6×10^6	100
	3×10^8	30

Reproduced from ref. 42.

formulae with the number of candidate interpretations remaining after the Heuristic DENDRAL program has applied its stored theory of chemical stability. The program constructs, using evidence of various kinds, a "GOODLIST" of substructures which must appear in any structure hypothesized by the program and a "BADLIST" of substructures which must not appear. As a simple example, at a given stage down a search tree might be the partial hypothesis $-\text{CH}_2-\text{O}-\text{CH}_2-$ and a possible next move for the structure-generator procedure might be to attach a terminal carbon, forming $-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_3$. But unless the data contains peaks at 59 and at M-15 this continuation is forbidden. Again, the structure-generator can be made to handle as a, "super-atom" a fragment indicated by the mass spectrum. Additional opportunities to do this arise when the presence of methyl super-atoms can be inferred from nuclear magnetic resonance data, when available.

Third, McCarthy's problem of the mutilated checkerboard³⁷ is quintessential to the point here discussed. The squares at opposite corners of an 8×8 checkerboard are removed, leaving sixty-two squares. Thirty-one dominoes are available, each of such a size and shape as to cover exactly two adjacent squares of the checkerboard. Can all the sixty-two

Table 1 A Labelling Scheme	
	1 Convex edge
	2 } Obscuring edges—obscuring body lies to right of arrow's direction
	4 } Cracks—obscuring body lies to right of arrow's direction
	6 } Shadows—arrows point to shadowed region
	8 Concave edge
	9 } Separable concave edges—obscuring body lies to right of arrow's direction—double arrow indicates that three bodies meet along the line

Reproduced from ref. 42.

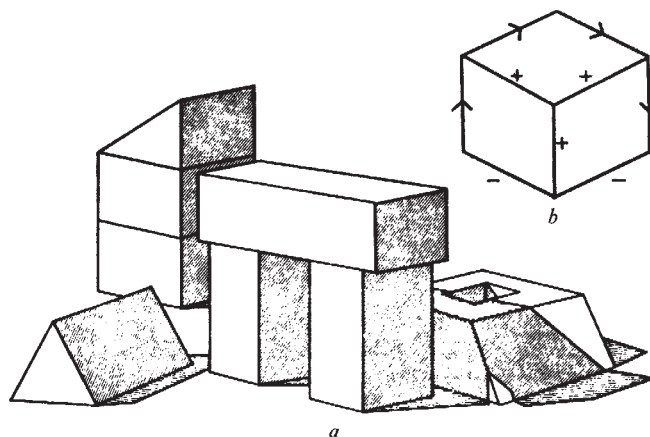


Fig. 3 a, A complex three-dimensional scene; b, Huffman labels for a cube. Plus implies a convex edge, minus implies concave, and an arrow implies that only one of the edge-forming surfaces is visible.

Table 3 Comparison of the Number of Topologically Possible Molecular Graphs Corresponding to Given Empirical Formulae with the Number of Candidate Interpretations Remaining after the Heuristic DENDRAL Program has Applied its Stored Theory of Chemical Stability

		Number of isomers	Number of inferred isomers	
			A	B
Thiol	1-nonyl	405	89	1
	n-decyl	989	211	1
	n-dodecyl	6,045	1,238	1
Thioether	di-n-pentyl	989	12	1
	di-n-hexyl	6,045	36	1
	di-n-heptyl	38,322	153	1
Alcohol	n-tetradecyl	38,322	7,639	1
	3-tetradecyl	38,322	1,238	1
	n-hexadecyl	151,375	48,865	1
Ether	Di-n-octyl	151,375	780	1
	bis-2-ethylhexyl	151,375	780	21
	di-n-decyl	11,428,365	22,366	1
Amine	n-octadecyl	2,156,010	48,865	1
	N-methyl-n-octyl-n-nonyl	2,156,010	15,978	1
	N,N-dimethyl-n-octadecyl	14,715,813	1,284,792	1

A, Inferred isomers when only mass spectrometry is used; B, inferred isomers when the number of methyl radicals is known from nuclear magnetic resonance data. Based on ref. 20.

squares be exactly covered by some tessellation of the thirty-one dominoes?

However sophisticated the search procedure which a heuristic program might use to attack this problem by trial and error, the combinatorics of the problem will defeat it. If the reader is unsure of this, let him mentally enlarge the board to, say, 80×80 , or $10^8 \times 10^8$. But so long as the dimensions of the board are both of even, or both of odd, length (such boards are called "even" boards) then the problem stays the same for any solver armed with certain crucial pieces of knowledge, namely: that the two squares which are removed from opposite corners of an even board must be of the same colour, and that each domino must cover exactly one white and one black square. The problem now falls apart. The mutilated checker-board cannot be covered.

To discover formal schemes within which such key facts can automatically be mobilized and their relevance exploited in an immediate and natural fashion is closely bound up with what was earlier referred to as "the representation problem". A familiar example is that certain representations of the game of Nim trivialize the calculation of a winning strategy; but the program capable of inventing such representations is yet to be devised.

Progress Towards an ICS

Two years ago I discussed in *Nature*²⁷ the possibility of implementing in software an Integrated Cognitive System (ICS). The attainment on a laboratory scale of a "working model", it was suggested, could be used as an indicator of ultimate feasibility. A working model of an ICS, as a minimal set of requirements, should be able: to form an internal representation of its task environment, summarizing the operationally relevant features; to use the representation to form plans of action, to be executed in the task environment; to perform directed perceptual sampling of the environment to switch execution along conditional branches of the plan; to recover from error state when execution fails; to cope with complex and ill-structured environments; to be told new goals and to work out its own approaches to them; and to use the record of past failures and successes to revise and extend the representation inductively.

A computer program which was not able to do most of the above, however excellent a feat of software technology it might be, would not count as an artificial intelligence program. The guidance software for the Apollo on-board computer, written for NASA by Draper Laboratories (J. Moore, privately

circulated report, Department of Computational Logic, University of Edinburgh) and charged with the task of getting the spacecraft to the Moon and back, is disqualified on this criterion. On the one hand, it is an acknowledged masterpiece, and on the other, in common with other and lesser automatic control systems, it scores a significant mark only for the third item in the above list.

The on-board computer does not need to plan because hand-coded routine have been provided for all probable situations—analogue, perhaps, to the elaborate, but essentially reflex, nervous system of an insect. The reason for regarding the Apollo on-board system as sub-intelligent is thus concerned with the nature of the internal model which it has of its environment. More than a quarter of a century ago Craik³⁸ first called attention to the crucial role in thought and perception of internal models. The world of the Apollo computer is so simple and determinate that its behaviour can be completely characterized by computationally simple equations. These equations, which comprise the system's "internal model" in Craik's sense, capture the dynamics of all possible configurations of the objects of its world, and supply all information needed about their interactions and properties.

But consider the mission: not to go to the Moon and back, but the much harder one of going down to the tobaccoist and back. By contrast with the space mission, the task environment is exceedingly complex and "messy" and the unexpected lurks at every point of the route (the stairs may be swept, unswept, blocked . . . , the front door may be open, shut, locked . . . , the weather may be bright, dull, wet, windy . . . and so on). Alternatively, and only a little less taxing (at least the environment does not contain other autonomous beings to worry about), consider the mission of a Mars Rover vehicle, such as that already envisaged by NASA³⁹ and by the space section of the USSR Academy of Sciences (N. Zagoruiko, personal communication). Arising from the fact that it is not possible to pre-program solutions to all problems which might arise while exploring an unknown terrain, a specific ten-year programme of machine intelligence research is regarded as a necessary preliminary condition for putting such operational vehicles into commission. Note that if such a vehicle is to handle all the tasks of autonomous exploration, and assembly and use of instruments, which will be demanded of it, then it must score seven out of seven on the criteria posed earlier.

That achievement lies in the future. How do matters stand today with regard to "working models"? Each of the seven capabilities listed can now be found in one or another experimental system, and there are some systems which exhibit many, or even most, of them. Unfortunately the most interesting capability of all, central to the phenomenon of intelligence, is the one which is still the least well understood—namely inductive generalization. Yet significant progress has been made^{16,40}.

In summary, incomplete systems are becoming commonplace and complete "working models", at the most primitive level, now seem not very far off. The likely technological lag before such systems might be upgraded to near-human intellectual performance is a topic for separate consideration.

Implications and Forecasting

It would plainly be desirable to find some objective basis for predicting the rate of development and social impact of machine intelligence. An objective basis is lacking at present and it is only possible to record samples of subjective opinion and to categorize lines of enquiry which more objective studies might follow. Fig. 4 summarizes some of the results of an opinion poll taken last year among sixty-seven British and American computer scientists working in, or close to, the machine intelligence field.

In answer to a question not shown in Fig. 4, most considered that attainment of the goals of machine intelligence would cause human intellectual and cultural processes to be enhanced rather than to atrophy. Of those replying to a question on the risk of ultimate "takeover" of human affairs by

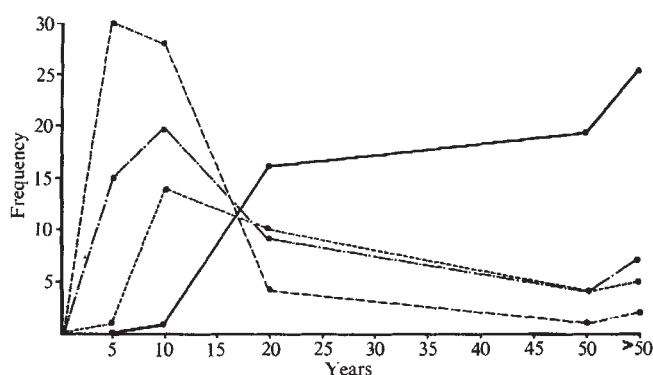


Fig. 4 Opinion poll on machine intelligence. Estimated number of years before: —, computing system exhibiting intelligence at adult human level; ---, significant industrial spin-off; — · —, contributions to brain studies; · · ·, contributions from brain studies to machine intelligence.

intelligent machines, about half regarded it as "negligible", and most of the remainder as "substantial" with a few voting for "overwhelming".

A working party recently convened under the auspices of the Rockefeller Foundation at Villa Serbelloni, Lake Como, on June 11 to 15, 1972, considered the gradations through which complex information systems might evolve in the future, ranging from contemporary industrial control systems, and "data look-up" retrieval, to autonomous computer networks developed for controlling urban functions (telephones, electricity distribution, sewage, traffic, police, banking, credit systems, insurance, schools, hospitals, and so on). The backbone of such systems will develop anyway, by straightforward elaboration of conventional computing technology, including the integration of the various computational networks into total systems. It seems likely that such systems will also ultimately incorporate autonomous planning and decision-taking capabilities, derived as "spin-off" from developments based on artificial intelligence in, for example, space and oceanographic robotics. A danger could then arise of city dwellers becoming dependent on systems which could no longer be fully understood or controlled. Counter-measures to such dangers might include the introduction of auditing procedures for computer programs, research on program-understanding programs, and system-understanding systems generally, and, finally, the advent of programs to teach the users of intelligent systems.

On the other side of the balance sheet, the working party took preliminary note of several anticipated benefits. The mechanization of industrial production has been associated in the past with the imposition of a deadening uniformity of design. Automated intelligence in the factory could offer the possibility of restoring the diversity and the "one off" capability originally associated with human craftsmanship. Related to this is the introduction of computer aids for the artist, composer, writer, architect and mathematician. Even the ordinary hobbyist might be enabled to perform feats which would today seem daunting or bizarre—building his own house, publishing his own writings, for example. The possible effects on computer-aided education have been stressed by others⁴¹: advances in this area will be of value not only to the young but also to older people as a means of acquiring new skills.

The formulation of an outline scheme of topics, and the compilation of relevant documents, represents an early stage of a study expected to occupy a number of years. Technical developments which occur in the intervening period will doubtless give such studies a firmer basis.

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Gravitational Waves and Relativistic Disks

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Relativistic disks enhance the intensity of radiation in directions near their plane. The gravitational pulses which Weber claims to have detected may arise in such a disk situated in the Galactic nucleus, with its plane aligned with that of the Galaxy. A typical enhancement factor is 30.

It seems improbable that an isotropic source of gravitational radiation situated at the Galactic centre can be responsible for the gravitational events observed by Weber¹; that would require embarrassingly high mass-loss rates (perhaps as high as $10^4 M_{\odot} \text{ yr}^{-1}$). Misner² has suggested that the radiation is produced by a gravitational synchrotron process, giving rise to narrowly beamed radiation confined to the plane of the Galaxy. But Davis *et al.*³ have concluded, on theoretical grounds, that such beaming would not occur to the required extent in the process envisaged by Misner. I describe here another model of anisotropic emission.

Relativistic disks are flat, strongly self-gravitating systems of particles or material, collapse of which is prevented by rapid rotation. As objects of possible astrophysical interest, they have been the object of much recent research⁴⁻⁶. My suggestion is that the Galactic nucleus contains a relativistic disk whose plane coincides with that of the Galaxy, and that gravitational radiation is generated at a "reasonable" rate within the disk. I show that the intensity is enhanced in directions near to the plane of the disk by the latter's gravitational field. A typical enhancement factor is ~ 30 , and possibly higher. The radiation might be generated in such a system by gravitational encounters between the particles (for example, neutron stars or black holes) composing it, or by accretion of these particles by a massive black hole at the centre of the disk. The latter possibility has an attractive feature, namely that Misner's mechanism, and the one described here would operate simultaneously, the two effects combining multiplicatively.

The Enhancement Factor

As an illustration, I consider a locally isotropic source of radiation situated at the centre of the disk. I use geometrical optics, which turns out to be reasonable if the radius R of the system $\gg 100 \times$ the wavelength λ of the waves detected by Weber, that is, $R \gg 2 \times 10^4 \text{ km}$. A ray leaving the centre C at a small angle α_i to the disk plane (see Fig. 1) can cross and re-cross the disk many times before escaping at an angle β to the plane; α_f is the crossing angle at the last crossing point P . (Transparency of the disk material to gravitational waves is quite justifiable, and in any case a disk composed of particles would almost certainly be optically thin.)

I make the not unduly restrictive assumption that the surface density of the disk material decreases with increasing distance from the centre, in which case the inequalities $\alpha_i > \alpha_f \gtrsim \beta$ hold (that is, regarding all angles as positive.) It is the fact that most rays have an escape angle β smaller than the starting angle α_i which is responsible for the intensity enhancement.

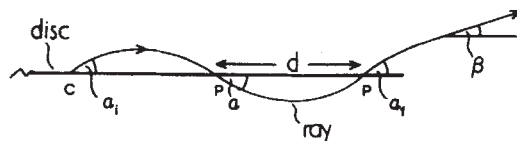


Fig. 1 A typical ray path.

Corresponding to a given β , there is a discrete set $(\alpha_{i,n})$ of values of α_i , each α_i corresponding to a given number n of crossing points. The smallest $\alpha_{i,n}$ (α_{min}) will correspond to the largest number n_{max} of crossing points, and to the smallest $\alpha_{f,n}$, which, from the above inequality, is approximately equal to β . The enhancement factor A is given by

$$A = \sum_{n=1}^{n_{max}} \left| \frac{d\alpha_i}{d\beta} \right|_{\alpha_i = \alpha_{i,n}}$$

In order to estimate A , I consider an infinitesimally thin Newtonian disk with surface density $\sigma(\rho)$, a function of the distance ρ from the centre. I shall give an indication of the modification to be expected from general relativity later. Ray paths are given by the orbits of particles moving with speed c . If z is the height above the disk plane, the local path of a radial ray crossing the disk at some point $\rho = \rho_0$ and small angle $\alpha = \alpha_0$ is given by

$$\frac{d^2 z}{dt^2} \sim -2\pi G(\sigma_0 + \sigma'_0 x)$$

and

$$\frac{dx}{dt} \sim c$$

where the prime denotes a ρ derivative, and $x = \rho - \rho_0$. Eliminating t between these equations, and integrating

$$z \sim \alpha_0 x - \frac{\pi G \sigma_0 x^2}{c^2} \left(1 + \frac{\sigma'_0}{\sigma_0} x \right)$$

The ray thus returns to the disk at

$$x \sim \frac{c^2 \alpha_0}{\pi G \sigma_0}$$

and at a crossing angle

$$\alpha_0 + \delta \alpha_0 \sim \alpha_0 \left(1 + \frac{1}{3} \frac{c^2 \alpha_0}{\pi G \sigma_0} \frac{\sigma'_0}{\sigma_0} \right)$$

The crossing angle α and distance d between crossing points can be treated as continuous functions of ρ if $d \ll R$, which is the case of interest here. I have $d \sim c^2 \alpha / \pi G \sigma$, and the above equation gives an estimate of α'

$$\frac{\alpha'}{\alpha} \sim \frac{\delta \alpha}{\alpha d} \sim \frac{1}{3} \frac{\sigma'}{\sigma}$$

giving

$$\alpha \sim \alpha_i \left(\frac{\sigma}{\sigma_i} \right)^{\frac{1}{3}}$$

and

$$d \sim d_i \left(\frac{\sigma_i}{\sigma} \right)^{\frac{2}{3}}$$

where the subscript i refers to values at $\rho=0$. These confirm that if $\sigma' < 0$, α diminishes as we move towards the edge of the disk, and d increases. The ray will escape at a value of σ corresponding to $d \sim R$, the final crossing angle being

$$\alpha_f \sim \alpha_i \left(\frac{d_i}{R} \right)^{\frac{3}{2}} \sim \alpha_i \left(\frac{c^2 \alpha_i}{\pi G \sigma_i R} \right)^{\frac{1}{2}}$$

This equation can be solved for $\alpha_{\min}$, the smallest α_i corresponding to a given β , which is given by $\alpha_f \sim \beta$. Thus

$$\alpha_{\min} \sim \left(\frac{\pi G \sigma_i R}{c^2} \right)^{\frac{1}{3}} \beta^{\frac{2}{3}}$$

The number of crossing points is $n \sim R/d_i \sim \pi G \sigma_i R / c^2 \alpha_i$, so that

$$n_{\max} \sim \left(\frac{\pi G \sigma_i R}{c^2 \beta} \right)^{\frac{3}{2}}$$

and

$$\alpha_{i,n} \sim \frac{\pi G \sigma_i R}{c^2 n}$$

In order to estimate $d\alpha_i/d\beta$, I note that when α_i changes from $\alpha_{i,n}$ to $\alpha_{i,n+1}$, β sweeps through a range $\sim \alpha_{f,n}$. Thus

$$\left| \frac{d\alpha_i}{d\beta} \right|_{\alpha_i=\alpha_{i,n}} \sim \frac{\alpha_{i,n} - \alpha_{i,n+1}}{\alpha_{f,n}} \sim \frac{1}{n^{\frac{1}{2}}}$$

Finally, I conclude that

$$A \sim \sum_{n=1}^{n_{\max}} \frac{1}{n^{\frac{1}{2}}} \sim 2 n_{\max}^{\frac{1}{2}} \sim 2 \left(\frac{\pi G \sigma_i R}{c^2 \beta} \right)^{\frac{1}{4}}$$

A has its maximum value of $2\beta^{-\frac{1}{4}}$ in the extreme relativistic limit, when $\pi G \sigma_i R / c^2 \sim 1$. The Solar System is about 10 kpc from the Galactic centre, and 4 ± 12 pc above the plane⁷, giving $\beta \sim 10^{-3}$ – 10^{-4} rad, and in the above limit $n_{\max} \sim 10^2$ – 5×10^2 and $A \sim 20$ – 40 . The mass-loss rate is reduced to roughly $300 M_{\odot} \text{ yr}^{-1}$, which is just about compatible with the observational upper limit of $200 M_{\odot} \text{ yr}^{-1}$ averaged over the past 10^8 yr obtained by Sciama, Field, and Rees⁸. This mass-loss rate is still high in the sense that a galactic mass ($\sim 10^{11} M_{\odot}$)

would disappear in 10^8 – 10^9 yr. An interesting feature of enhancement factors ~ 30 is that the mass loss per event is reduced to about one solar mass. The requirement $d_i \gg \lambda$ when $\alpha_i = \alpha_{\min}$ gives the geometrical optics condition $R \gg \alpha_{\min}^{-1} \lambda \sim 100 \lambda$ in this case.

The above result would allow $A \rightarrow \infty$ as $\beta \rightarrow 0$. But the finite thickness T of a real disk would intervene to prevent this, because for sufficiently small values of α rays would not rise out of the disk material, and would only enter vacuum on reaching the rim of the disk. The height $z_{\max}$ to which a ray rises above the plane is given by

$$z_{\max} \sim \frac{c^2 \alpha^2}{4 \pi G \sigma}$$

The requirement that this be greater than $T/2$, when $\rho=0$ and $\alpha = \alpha_{\min}$, gives $T/R < \beta/A$ as an approximate sufficient condition for the finite thickness not to be the limiting factor. Thus only thin disks ($T/R < 10^{-5}$) can lead to the amplifications mentioned above. Such systems would almost certainly be unstable with respect to fragmentation⁶, but probably only in the sense that flat galaxies develop a spiral structure. The thickness is determined by the particle mean square velocity v^2 perpendicular to the disk plane, the relationship being $T/R \sim v^2/c^2$ in the extreme relativistic limit. Thus $v/c < 3 \times 10^{-3}$.

A possible objection to the model is that Weber detects pulses of radiation, with pulse length⁹ < 0.5 s. The different path lengths involved here would tend to smear out a pulse; the overall path difference is of order $\alpha^2_{\min} R \sim A^2 \beta^2 R/4$. Taking $\beta = 3 \times 10^{-4}$ rad and $A = 30$, the above pulse length gives an upper limit of about 10^{10} km for the radius R . The corresponding upper mass limit is of order $3 \times 10^9 M_{\odot}$.

General Relativistic Modifications

The solution of Einstein's vacuum field equations which describes the gravitational field of a static infinitesimally thin homogeneous sheet, situated at $z=0$, is the metric

$$ds^2 = Z^{-2} dt^2 - Z^4 (dx^2 + dy^2 + dz^2)$$

where $Z = 1 - 2\pi\sigma|z|$ (units in which $G=c=1$ are used here). This is a form of the Kasner solution¹⁰. The event horizons at $z = \pm 1/2\pi\sigma$ correspond in the Newtonian case to surfaces from which falling particles would reach the sheet with the velocity of light. It is instructive to compare null geodesics in this space-time with the corresponding Newtonian ray paths. A null geodesic in the plane $y=0$, leaving the origin at angle α to the sheet, obeys the equation

$$\frac{dx}{dz} = \frac{\cos \alpha}{(Z^6 - \cos^2 \alpha)^{\frac{1}{2}}}$$

When α is small, which is the case of interest here, we have $2\pi\sigma|z| \leq \alpha^2/6 \ll 1$. This equation then integrates to

$$x \sim \frac{1}{6\pi\sigma} (\alpha - (\alpha^2 - 12\pi\sigma z)^{\frac{1}{2}})$$

so that the ray returns to the sheet at $x \sim \alpha/3\pi\sigma$. The maximum height above the plane is $z_{\max} \sim \alpha^2/12\pi\sigma$. Comparing these with the Newtonian values, Newtonian theory underestimates the pull of the sheet by a factor of 3.

Another non-Newtonian effect which has been ignored is that due to the rotation of the disk. This would make the rays spiral out from the centre, and thus increase the effective radius of the disk.

These considerations suggest that the Newtonian enhancement factors are somewhat pessimistic.

It is difficult to know what to call a reasonable mass-loss rate for the Galaxy in this context, but $10^{10} M_{\odot}$ over 10^9 yr would probably be regarded as a reasonable upper limit. The largest enhancement factor of 40 presented here, which reduces the observed rate to about $250 M_{\odot} \text{ yr}^{-1}$, is thus too small by about an order of magnitude. Exact general relativistic calculations of the effect will probably reveal that the figure of 40 is an underestimate. But perhaps the most promising avenue for further research is the possibility of combining this effect with another one, such as that described by Misner.

Lawrence¹¹ has proposed a related model, and invokes flattened rotating objects (spinars) to produce the enhancement. But I believe his treatment to be inadequate, and in any case there are significant qualitative differences between his effect and the one described here. In particular, a thin disk, and the associated "multiple crossing" phenomenon described above, are essential for this effect.

I thank Professor Martin Rees for pointing out an error in an earlier version of this paper, and for subsequent valuable

conversations. I thank the referee for bringing the work of Lawrence to my attention.

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Measurements of Some Hydrogen-Oxygen-Nitrogen Compounds in the Stratosphere from Concorde 002

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Measurements of the sub-millimetre wavelength far infrared emission spectrum of the stratosphere were made from Concorde 002 during the tour of the Far East in June 1972. The spectra have been analysed to provide concentration data for the species H_2O , O_3 , HNO_3 , N_2O , and NO_2 .

PREVIOUS high resolution measurements¹⁻³ of the sub-millimetre infrared emission spectrum of the stratosphere have shown that, in addition to lines of H_2O , O_2 and O_3 , other lines may be observed which are due to species such as HNO_3 , NO_2 , N_2O and SO_2 , particularly at large air masses. The importance of such observations is clearly very great in view of the present scientific debate on the physics and chemistry of the stratosphere, and the possible effects of high-flying aircraft. The work reported here is the first of a series of measurements to determine the concentrations of several of the species of interest, from the far infrared spectrum.

The basic technique^{1,2,4} is to use a Michelson interferometer together with a helium-cooled Rollin bolometer, the whole system having a spectral response in the $300 \mu\text{m}$ to $3,000 \mu\text{m}$

range. The advantages of this system over others when making atmospheric observations have been previously identified^{1,2,4,5}.

Apparatus and Data Collection

The apparatus was a modified version of that flown in a Comet 2 previously^{1,2}, and was installed in Concorde 002 in April 1972. Particular modifications which had to be made included: the manufacture of a special polypropylene window to be fitted into the fuselage in one of the passenger window apertures; the design and construction of a new data logging and control system, for faster scanning of the interferometer than had been used previously; and change of the zenith angle to 90° , in order to increase the air mass viewed by the apparatus. The use of a Cassegrain telescope was abandoned in favour of a radiation collecting horn, with a 5° (total) field of view.

Data were recorded on paper tape and stored for processing. The resolution of the interferometer was 0.067 cm^{-1} (unapodized⁶) and a single interferogram was recorded in 11 min. The equipment, once started, operated automatically, with the scanning motion of the interferometer mirror being reversed by micro-switches.

During the Far East tour, interferograms were recorded wherever possible subject to the following two criteria: first, that the aircraft was flying at sufficiently high altitude to yield stratospheric data; and second, that liquid helium was available. Supplies of liquid helium were arranged through asso-

ciate companies of the British Oxygen Company Ltd, and with Air Products Ltd. Some fifty-five interferograms were recorded of which thirty were finally used to provide data: the others were rejected because of unsuitable atmospheric conditions, excessive aircraft manoeuvring or because of serious noise interference.

The tour went through Iran, to India, Singapore and Japan; then on to Australia (most of the time being spent in S Australia), and returning *via* Singapore, India and Saudi Arabia. It started on June 2, and the last set of observations was made on June 28 (Table 1). Some data for the UK were recorded before the tour, on shakedown flights.

Sample Spectrum

In Fig. 1 I show a sample spectrum (20/6.3+4 in Table 1) which is the average of two 11 min runs recorded over the Sydney (Australia) area on June 20, 1972. The spectrum has been labelled with assignment numbers, and a partial list of

curved atmosphere model^{15,16} was used to derive the total air mass (in cm atmos) with which to produce the volume mixing ratio, V , for each species. This is, of course, certainly not true for several of the species: however, vertical distributions of mixing ratio were not the prime concern of this work, and will be considered later. This mixing ratio therefore is an effective mean value (averaged over height) for each species. For the gases emitting in the weak region of the curve of growth (HNO_3 , NO_x , weak O_3) the scale height is the atmospheric scale height. For H_2O and strong O_3 lines the scale height is smaller (~ 2 km in the vertical for H_2O). Both column density and average mixing ratio are listed in Table 3.

Global Data

Individual values of volume mixing ratios then derived in this way for H_2O , HNO_3 , N_2O , and O_3 , are given in Table 1, where I also give details of the flight level, tropopause level,

Table 1 Volume Mixing Ratios (Assumed Constant with Height)

Run identifier	VHNO_3 $\times 10^9$	VH_2O $\times 10^6$	VN_2O $\times 10^9$	VO_3 $\times 10^6$	Flight h (1,000 foot)	Altitude (mbar)	Tropo- pause h_t (1,000 foot)	Altitude (mbar)	$h-h_t$ (1,000 foot)	Approximate latitude and longitude	
30/5.1	2.0	3.93	240	4.9	48	140	38	230	10	48N	08W
9/6.2+3	3.1	5.50	300	4.6	50	120	52	105	-2	01N	104E
11/6.1+2	3.4	6.60	275	4.6	48	125	50	115	-2	05N	110E
11/6.3+4	2.8	6.22	270	4.8	51.5	110	50	115	1.5	10N	115E
14/6.1+2	3.2	6.55	325	4.6	47	145	52 to 55	85 to 105	-5 to -8	35N	140E
15/6.2	2.9	5.70	270	5.5	44.5	155	52.5	100	-8	30N	135E
15/6.5	2.7	6.80	365	5.1	48	130	53	95	-5	18N	121E
20/6.1+2	3.7	5.90	265	4.8	45	150	37.5	230	7.5	35S	151E
20/6.3+4	3.0	3.60	190	3.7	49	120	37.5	230	11.5	35S	151E
20/6.6+7	1.9	2.85	215	4.8	47.5	130	37	235	10.5	37S	149E
20/6.8	2.7	3.60	215	3.7	40	195	37	235	3	40S	145E
22/6.2	2.1	3.15	210	4.9	49	125	39.5	200	9.5	40S	145E
22/6.3	3.0	5.62	350	5.4	40	195	39.5	200	0.5	40S	145E
23/6.1+2	2.5	2.49	240	4.0	52	105	37	235	15	30S	140E
23/6.3+8	2.7	10.2	390	5.9	40	190	52.5	100	-12.5	20S	133E
23/6.12+13	2.5	9.21	250	3.9	47	145	54	90	-7	12S	125E
23/6.14+15	3.2	8.75	245	4.9	52	105	52.5	100	-0.5	05S	115E
24/6.3	1.9	12.9	270	3.7	44	155	*	—	—	01N	104E
28/6.1+2	3.5	16.1	250	3.4	35	260	52.5	100	-17.5	20N	70E

* No meteorological data available.

assignments is given in Table 2 for the species HNO_3 , N_2O , NO , NO_2 and SO_2 . The complete list including H_2O , O_2 and O_3 may be found elsewhere⁷. The lines of HNO_3 and NO_x which have been used for concentration determination are marked in Fig. 1 by an asterisk (Table 2).

The spectrum was assigned, using the compiled data⁷, and the equivalent widths of the lines of interest were measured manually and tabulated. For H_2O and O_3 a technique^{4,8} of deriving the mixing ratio relative to O_2 emission lines was used. For the other species, which are in the weak region of the curve of growth and so cannot simply be compared with strong O_2 emission lines, laboratory measurements⁹⁻¹² and calculations^{13,14} have been used to calculate the equivalent path length of the species above the aircraft. A correction for the temperature of the stratosphere (assumed to be 225 K) was made, and the equivalent path expressed in units of cm atmos. The data were then treated in two ways. First, the concentrations were converted to units of column density, cm^{-2} (that is, the number of molecules above the flight level in a column of 1 cm^2 cross-section). Second, it was assumed that the mixing ratio was constant with height, and a standard

and approximate geographical location. An estimate for the total amount of NO_2 has also been made⁶, but the spectral assignments are somewhat uncertain for this species (see below), and so no results have been given in Table 1.

In Table 3 I give the global averages for the data, with an estimate for the spread in values obtained. The errors involved in this spread will include random errors in equivalent width measurement (including the effect of noise in the spectrum), fluctuations in air mass due to aircraft roll and manoeuvring, and real variations due to height, h , tropopause height h_t , or latitude change. The data also include a contribution due to systematic errors in source spectroscopic data⁹⁻¹⁴, which have been considered elsewhere⁷. The systematic errors are due to poor signal-to-noise ratios in the laboratory spectra, incorrect transmission coefficient estimates, and lack of theoretical data on line strengths and half widths.

Total Concentrations and Mixing Ratios

For my present purposes I wish to report only on measured total concentrations and mean mixing-ratios, as the first stage

in this programme of work. But I note that in several cases, the behaviour of volume mixing ratio as a function of height indicates variations which are possibly significant. Fig. 2 shows examples, for H_2O , N_2O and HNO_3 , of data taken from Table 1. The lines have been hand-drawn through the points. These trends are being considered and I hope that vertical distribution profiles will be evaluated, together with further measurements at several zenith angles.

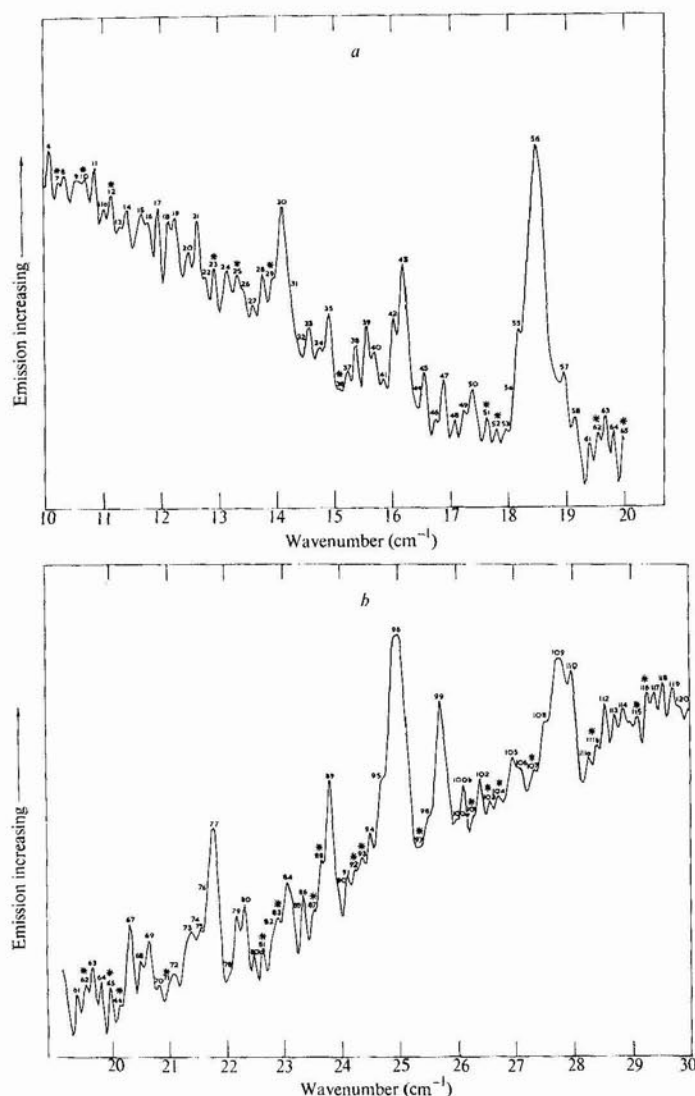


Fig. 1 The average of two spectra at a resolution of 0.067 cm^{-1} . The numbers refer to the full assignment table to be found in ref. 7: a partial list is given in Table 2 for the asterisked lines, which are those due to HNO_3 , N_2O , NO_2 and SO_2 . a, $10\text{--}20\text{ cm}^{-1}$; b, $20\text{--}30\text{ cm}^{-1}$.

I now summarize the results obtained to date, with a brief species-by-species discussion, and compare these with measurements and calculations by other workers.

For HNO_3 , values fairly closely grouped around a mean mixing ratio of 3×10^{-9} are obtained. No significant latitudinal variation is observed (Table 1), but some evidence of a non-linear vertical distribution may be derived from the behaviour with height above the tropopause (Fig. 2). HNO_3 has previously been observed by Murcay *et al.*¹⁶, and Rhine *et al.*¹⁷ have interpreted their results to be equal to an average mixing ratio of about $3.2 \pm 30\% \times 10^{-9}$, with an "ozone-like" distribution. Lazrus *et al.*³⁰ obtain values between 2 and

4×10^{-9} at 20 km. Recently Murcay (private communication) has obtained values of 4.5×10^{-9} by volume at about 23 km, with about 1×10^{-9} at 15 km.

N_2O has been measured in the troposphere and the stratosphere by a number of workers, because of its importance in the nitrogen cycle. Schutz *et al.*¹⁹ obtain very similar results to those of Table 3, of 250×10^{-9} at the tropopause and 220×10^{-9} at about 15 km. Goldman *et al.*²⁰ obtain a distribution of similar shape, but with lower absolute values, reporting 150×10^{-9} at the tropopause. Bates and Hays²¹ have considered the factors affecting the concentration of N_2O in the stratosphere, and present a number of profiles depending on the adopted value of the vertical exchange coefficient K_z ($\text{cm}^2\text{ s}^{-1}$). My results indicate, using Fig. 3 of Bates and Hays, a best fit with K_z equal to $1 \times 10^3\text{ cm}^2\text{ s}^{-1}$.

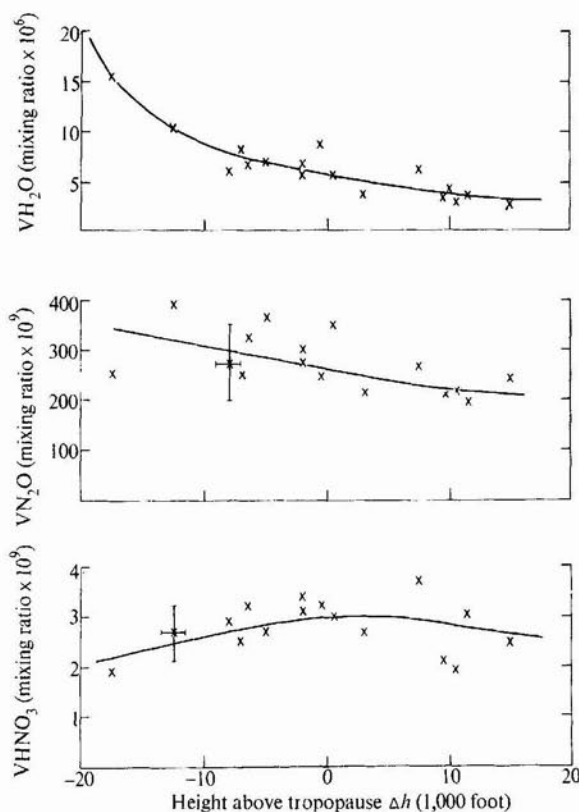


Fig. 2 A compound diagram showing the variation of VH_2O , VN_2O and VHNO_3 with height above the tropopause. The curves are hand drawn through the data points and the significance of the variations is discussed in the text.

In the case of NO_2 my results give about 20×10^{-9} by volume. As mentioned above, however, the accuracy of this measurement is severely limited as the spectral assignments are somewhat uncertain (lines 71 and 83 in Table 2) and, in addition, source spectroscopic data are of poor quality. More work is currently being done on NO_2 , and it is hoped that we will be able to make a more definite statement regarding this species soon. The only other estimate is one by Ackerman and Muller²³, who have used data obtained by Murcay and co-workers, from a balloon at 32 km, as well as their own data, to produce a profile of NO_2 which varies between 10^{-9} and a maximum of about 10×10^{-9} centred at about 28 km. This estimate is considerably lower than mine. But until the accuracy of source spectroscopic data has been established in both the spectral bands it is probably more meaningful to comment on the similarity of the results. The

Table 2 Partial List of Assignments

Line number	Measured wavenumber (cm ⁻¹)	Predicted wavenumber (cm ⁻¹)	Species
7	10.181	10.25	HNO ₃
10	10.650	10.65	HNO ₃
		10.72	N ₂ O
12	11.064	11.08	HNO ₃
23	12.963	12.96	HNO ₃
25	13.200	13.14	HNO ₃
29	14.10 (sh)	13.94	HNO ₃
36	15.094	15.06	N ₂ O
51	17.593	17.58	N ₂ O
52	17.70	17.75	HNO ₃
62	19.566	19.566	SO ₂
65	19.988	20.09	N ₂ O
66	20.172	20.26	NO ₂
		20.26	HNO ₃
71	20.944	21.01	NO ₂
81	22.578	22.59	N ₂ O
		22.62	HNO ₃
83	22.96 (sh)	22.89	NO ₂
87	23.59 (sh)	23.45	N ₂ O
88	23.71 (sh)	23.67	HNO ₃
92	24.262	24.18	NO ₂
		24.26	N ₂ O
93	24.375	24.45	HNO ₃
97	25.381	25.45	NO ₂
		25.48	SO ₂
101	26.287	26.14	HNO ₃
103	26.609	26.54	HNO ₃
104	26.766	26.73	NO ₂
		26.76	N ₂ O
107	27.325	27.39	HNO ₃
		27.339	O ₃ (weak)
111b	28.47	28.44	NO
		28.50	SO ₂
115	29.119	29.05	HNO ₃
116	29.280	29.28	N ₂ O
		29.29	NO ₂

sh=Shoulder; full list of assignments given in ref. 7.

column density of NO₂ which I have calculated approximately from Ackerman and Muller's data is 6.5×10^{15} cm⁻², to be compared with my result of about 5×10^{16} cm⁻². The latter figure will be revised in the near future once improved source data is available.

Table 3 Global Average Stratospheric Mixing Ratios

Species	Column density (mol cm ⁻²)	Average volume mixing ratio	Comments
H ₂ O	7.7×10^{18}	$(3.0 \pm 0.3) \times 10^{-6}$	Rapid increase below tropopause. Constant up to 60 km
O ₃	1.2×10^{19}	$(4.8 \pm 1.0) \times 10^{-6}$	Naturally variable
HNO ₃	7.2×10^{15}	$(2.8 \pm 0.7) \times 10^{-9}$	
N ₂ O	5.4×10^{17}	$(210 \pm 50) \times 10^{-9}$	Measured 3 km above tropopause
	7.0×10^{17}	$(270 \pm 60) \times 10^{-9}$	Measured at the tropopause

For water vapour our group has made a large number of observations over several years^{4,5,29} which agree well with the present result of 3×10^{-6} by volume in the stratosphere, constant with height up to about 60 km²⁹. Houghton *et al.*²⁴ have obtained results around 5×10^{-6} , and Mastenbrook²⁵ quotes values of $3-4 \times 10^{-6}$. Our estimates for H₂O should be accurate to better than $\pm 10\%$ (refs. 4, 5). Shimazaki and Laird²⁶ calculate that at about 70 km the mixing ratio is about 3×10^{-6} , and decreases at higher altitudes.

The result I obtain for ozone is, as for the other species,

averaged over altitude, tropopause height and location, and so is an average value which does not reflect any of the known seasonal trends. It would, for instance, have been interesting to observe at more southerly latitudes than 40° S in order to attempt to detect any polar increase. But the average value I obtain is in good agreement with known values²⁷.

Because the O₃ amount is known to vary considerably with time and location, and because some of the species of interest are thought to be associated with O₃ (for example, HNO₃, NO₂), it is at least possible that these species will exhibit similar fluctuations. In this sense, again, any real variations will tend to be lost in the imposed variations of height, latitude, and tropopause height necessary during this experiment (Table 1).

Finally, though I have not quoted a result in Tables 1 and 3, I mention a first estimate which has been made for the mixing ratio of SO₂: this result, which is based on the tentative assignment of line 62 to SO₂, is approximate, and confirmation will have to await more accurate source data and better experimental resolution. But at present I obtain an upper limit of 3.5×10^{-9} for VSO₂.

Many people have been involved in these experiments. I list significant group leaders. At the NPL, Mr N. R. Swann designed and built the control electronics, and assisted in the experiment. Dr N. W. B. Stone also helped.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Rotation of the Earth from AD 1663–1972 and the Constancy of G

TIMINGS of 40,000 lunar occultations of stars from 1943–1972 have recently been analysed to determine improvements to the Moon's orbital elements, and difference between the ephemeris (ET), universal (UT) and atomic (IAT) time scales. Full details are to be published elsewhere, but the analysis for the period for which the atomic time scale is available are of immediate interest. They can be used in conjunction with results of earlier analyses to give a clearer picture of variations of the rate of rotation of the Earth and to provide evidence that is relevant to whether the constant of gravitation, G , is variable.

For 1955.5 to the present, the variations in the rate of rotation of the Earth are given by the difference between UT and IAT. The observed times of occultations of stars by the Moon were therefore expressed on the scale IAT + 34.24 s (an approximation to the particular ephemeris time scale, ET2, which is the time argument of the currently-adopted lunar ephemeris¹) and used in a least squares analysis to determine corrections to the orbital elements on which the current lunar ephemeris is based. The resulting correction to the expression for the mean longitude of the Moon is (in arc s)

$$\Delta L = -(0.08 \pm 0.02) + (1.4 \pm 0.1)(T - 0.63) - (10 \pm 3)(T - 0.63)^2 \quad (1)$$

where T is the time in centuries from 1900. We can interpret this as arising from the difference between ET2 and (IAT + 32.24 s); thus since the mean longitude of the Moon increases by 1 arc s in 1.821 s

$$\text{ET2} = \text{IAT} + (32.39 \pm 0.04) - (2.55 \pm 0.18)(T - 0.63) + (18.21 \pm 5.46)(T - 0.63)^2 \quad (2)$$

This implies that, at 1663.0, IAT + 32.39 s would be a better approximation to ET2 and that the second of the ET2 scale is equal to $(1.0 - 0.8 \times 10^{-9})$ SI seconds, or $(9, 192, 631, 762 \pm 1)$ cycles of the defining caesium transition. The quadratic term could be interpreted as arising from a fundamental linear increase of the duration of the ephemeris second implied by gravitation theory with respect to the SI second, but it is more reasonable to attribute it to an error in the earlier correction [see equation (3)] applied to the lunar theory of Brown² to reduce the time argument to agree with ephemeris time defined by Newcomb's³ theory for the Sun. The time scale obtained by applying this further correction to the mean longitude is here denoted by ET*: it is a dynamic time scale that corresponds as closely as possible to the atomic time scale over the common period.

The previously tabulated values of $\Delta T = \text{ET2} - \text{UT}$ should be corrected by the value of $\text{ET2} - (\text{IAT} + 32.24)$ from equation (2) in order to give a better representation of the variations in the rate of rotation of the Earth. Applying this correction to the annual mean values given by Martin⁴ for 1663–1860, by Brouwer⁵ for 1861–1949 and in the Astronomical Ephemeris⁶ for 1950–1972, we obtain the differences, $\Delta T^* = \text{ET}^* - \text{UT}$, shown in Fig. 1A. The accumulated time difference, ΔT^* s

is caused by variations in the length of the day with respect to the length of the mean solar day near 1900.

The curve in Fig. 1A is of a parabolic form (corresponding to a gradual increase in the length of the day, due to tidal friction), but it is better represented by three parabolic arcs of steady deceleration of the rate of rotation of the Earth, linked by shorter periods of rapid acceleration, together with fluctuations about these mean rates. Representative values of the excess of the length of the day in ms with respect to the day near 1900 are given by the first derivatives along the dashed curve in Fig. 1A and are shown in B.

I have looked for, but have been unable to find, any differences in the interpretations or methods of analyses of the data which could have led to the major departures from a constant rate of deceleration as shown in Fig. 1. It is therefore necessary to find some cause (such as differential motion at the core-mantle interface^{7,8}) to account for the large fluctuations.

The adopted coefficient $(+7.14'')$ of T^2 in the theory of the Moon's motion due to Brown is a theoretical value that takes into account the gravitational and precessional effects, but

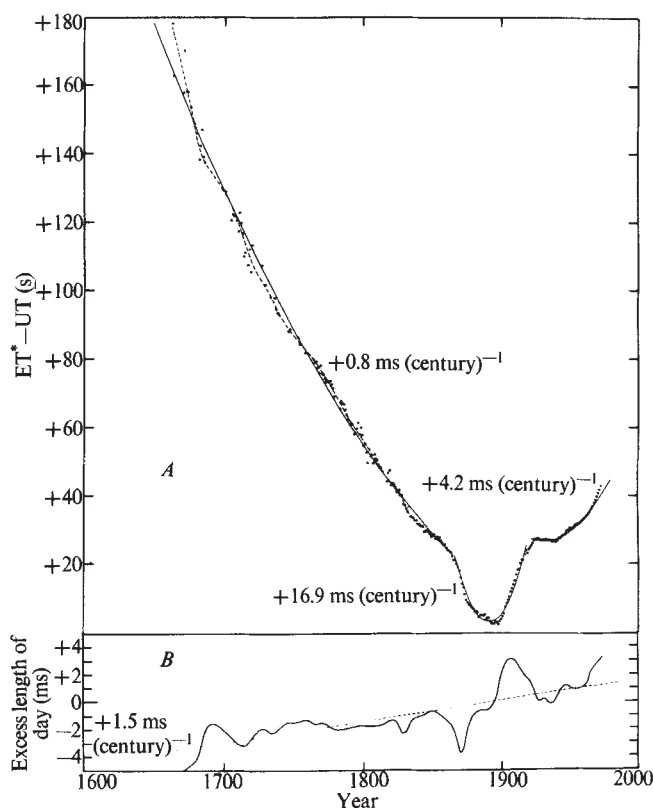


Fig. 1 A, Accumulated time difference on an adjusted ephemeris time scale, ET*, due to variations in the length of the day relative to the reference day near 1900. The three solid curves correspond to constant rates of increase in the length of the day. B, Excess length of the day compared with the day near 1900. The curve is the first derivative of A. The dashed line is the average rate of increase of the length of the day.

does not make any allowance for tidal interactions in the Earth-Moon system. The lunar ephemeris is now based on the value $-4.08 T^2$ which follows from the addition (first proposed by Clemence⁹) of the correction

$$\Delta L = -8.72 - 26.74 T - 11.22 T^2 \quad (3)$$

which was deduced from analyses by Spencer Jones¹⁰ of observations of the Sun, Moon and planets from 1700-1930. Thus, combining equations (3) and (1), we obtain a term $(-21 \pm 3'') T^2$ for the observed additional (tidal) deceleration of the Moon in mean longitude with respect to atomic time from 1955.5-1972.0. This supports the result of Van Flandern¹¹ of $(-26 \pm 8) T^2$ from occultations, and is in good agreement with the value of $(-19 \pm 4) T^2$ obtained by Oesterwinter and Cohen¹² who analysed meridian observations of the Sun, Moon and planets for the period 1912-1968. It also agrees with a value of about $(-19 \pm 3) T^2$ deduced from ancient solar eclipses^{13,14} with a mean epoch around AD 0.

Assuming transfer of angular momentum in the Earth-Moon system and adding a part for the Sun's tidal torque, a value of $-20 T^2$ corresponds to a rate of change of $+4 \text{ ms (century)}^{-1}$ in the length of the day. On the other hand, the value of ΔT ($\sim +4.5 \text{ h}$) determined from the eclipse data gives an average rate of only $+2 \text{ ms (century)}^{-1}$. The average rate for the period 1663-1972 from Fig. 1 is $+1.5 \text{ ms (century)}^{-1}$.

Thus we are faced with two difficulties. First, why does Spencer Jones's result of $-11 T^2$ differ so much from the other results? Second, what causes the implied average non-tidal rate of change of $-2 \text{ ms (century)}^{-1}$ in the length of the day?

Spencer Jones's result is strongly dependent on the analysis of times of transit of Mercury across the Sun, and Williams¹⁵ and Clemence¹⁶ have confirmed his values. A possible explanation of the first problem is that Mercury has a deceleration of $(-3.1 \pm 0.9) T^2$ in mean longitude which does not arise from the gravitational theory of its motion.

Hoyle¹⁷, in his recent presidential address to the Royal Astronomical Society, however, has suggested that both anomalies can be resolved if the constant of gravitation, G , varies inversely with time, provided that the value of the Moon's deceleration from ancient eclipses is interpreted as including both tidal and non-tidal (that is, depending on $\dot{G}$) components. There is little doubt that my value obtained directly with reference to IAT does so; but there is doubt about that from the ancient eclipses. It seems to me that the value of the deceleration obtained from ancient eclipses is measured with respect to a gravitational time scale, and that any variation of G with respect to an atomic time scale will not be apparent. If this argument is valid, the deduced value includes only the tidal component. It follows that the close agreement of this value with that deduced with respect to IAT suggests that $G=0$, or, allowing for an uncertainty of $\pm 3 T^2$, $\dot{G}/G \lesssim 2 \times 10^{-11} \text{ yr}^{-1}$. (The relationship between the units in Hoyle's paper and those above are as follows. If d is the rate of change in the length of the day in ms (century)^{-1} , $\dot{\omega}/\omega = -0.12 d \text{ aeon}^{-1}$, where an aeon is 10^9 yr : $\dot{h}$ is twice the coefficient of T^2 .) An extension of the work of Spencer Jones to include observations since 1937 is urgently required.

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The Orbit of Halley's Comet

RECENT investigations by Brady¹ have yielded the suggestion that the historical observations of Halley's Comet can be interpreted as indicating the existence of a large planet beyond Pluto having an orbital period of 464 yr. One of us² has carried out a detailed study of the past orbit of Halley's Comet using an extension of Cowell and Crommelin's method of Variation of Elements. Either Kiang's material² was capable of showing Brady's result but it had not been noticed; or his calculations were insufficiently accurate; or Brady's result was better accounted for by another explanation. We have attempted further calculations to decide between these alternatives, and we now believe that the third alternative is correct.

Brady first found that, by comparison with the observed times of perihelion passage, the theoretical motion of a body following the orbit of Halley's Comet was much improved, after full allowance was made for the gravitational effects of the planets, by incorporating a small empirical term in the equations of motion: explicitly this term was in the nature of a secular change in the central mass (the Sun). But from the kinematic point of view, an almost exact equivalent would arise from successive tangential impulses to the motion which might, as far as could be told from the observations, occur at or near the time of perihelion passage. Kiang's average "non-gravitational" change of mean motion over successive returns had been thought to be caused by a recurrent tangential impulse near perihelion passage arising from the interaction between reaction from solar heating and rotation of the comet nucleus. Such non-gravitational effects were the basis of evidence originally put forward by Whipple³ in support of his ice-rock agglomerate model of comets. Further, the size of Brady's empirical term is just that which would account for the average change in mean motion per revolution previously calculated, and if the minor modifications in recorded perihelion-passage times, which had been evaluated as an intrinsic part of Kiang's investigation, are incorporated into Brady's orbital comparisons where he used his empirical term, the already small residuals found by him would be further reduced, rather markedly. Without the benefit of this extra encouragement, however, Brady was not wholly satisfied with his empirical term and he therefore sought an explanation from gravitational effects of an unknown "Planet X", especially when he found that the residuals in perihelion passage behaved in a quasi-sinusoidal way with a period of about 600 yr if the empirical term were omitted.

We therefore turned our attention to the question of how a long periodicity could arise in the departures from a theoretical orbit through the effect, say, of a tangential impulse occurring once per revolution of the comet in its orbit, that is, every 76 yr. That such an apparent periodicity is possibly an inherent tendency is indicated by Fig. 1 of ref. 1 which shows the effect on the expected time of perihelion passage due to neglect of the gravitational attraction of Pluto. This, with a small amplitude, shows a quasi-period of about 600 yr, even though Pluto's period is 248 yr.

We considered a simplified numerical model consisting of the Sun, Jupiter and Halley's Comet. Full details will be published later; in brief, we found that Brady's quantity ΔT (departure from predicted time for a particular orbital position) can be represented as governed by a linear second-order differential equation in which the coefficients are periodic with the periods and beat periods of Jupiter and Halley's Comet. There is a formal resemblance between this equation and Hill's equation⁴ in lunar theory and we expect that it will be possible to show from the analysis how a dominant long-period term can emerge. Numerical solutions of the equation have been found to show a long-period variation (600–700 yr) for a range of initial conditions. We believe that any error, whatever periodicity it may have, will propagate with a long periodicity, the exact value of which is an intrinsic property of the 3-body configuration.

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The Geophysical Consequences of Professor Lyttleton

HAD Professor Lyttleton sent me his letter¹ before publication, I would have suggested that he replaced his homely parable with a straightforward account of how his controversy with me has—unhappily—arisen. To judge from his recent review², from which the quotations I will use are drawn, he has become seriously displeased that his attempts to revive Ramsey's theory³, that the Earth's core is not iron but a phase change undergone by ferromagnesian silicates of the mantle at high pressure, has not “for some curious reason . . . found much favour with geophysicists”. He extended Ramsey's theory by postulating that the pressure at which this hypothetical phase change occurs decreases with increasing temperature. Thus starting with a cold accreted Earth—wholly of silicate with no iron core—the disseminated radioactivity heated the Earth up to the temperature at which the phase change to a denser state began at the centre. Thus the core grew and the Earth's radius contracted.

Using an empirical relation between bulk modulus and pressure for the Earth's mantle and core at the present time, which seismology provides, along with the laws of gravitation and mechanical equilibrium, Professor Lyttleton produced a host of models of the density distribution and constitution of the evolving Earth with various sizes of the growing core and of the Moon and each terrestrial planet². For the parameters of these models² he claims “considerable numerical precision”. I note, in passing, that the empirical relationship at the basis of his computations is not a true equation of state as it does not include temperature.

His conclusion that the Earth's radius has decreased by about 300–400 km during its life, which he announced as a theory of mountain building⁴ (the classical theory of the shrinkage of the Earth's crust by the supposed cooling of the interior had been judged quantitatively inadequate), unfortunately appeared just as geologists and geophysicists had come to take seriously the great variety of new evidence for continental drift⁵. The contractions of the crust in mountain building began to appear as a second order effect compared with the great displacement of the continental blocks, rather than as the fundamental problem of tectonics.

Professor Lyttleton², much concerned, sought to warn geophysicists against “their transport of enthusiasm for the postulates of convective and other motions that are the main concern of geophysics today”, and helpfully tried to steer them away from unproductive researches: “For what becomes of the theory so created (of those postulated motions, such as continental drift) if the phenomenon itself fails to materialize?” and alerted them to the possibility that the whole question might lead to a *reductio ad absurdum*: “are all the continents moving, or are some fixed on the Earth and some moving . . . What happens when all the continents come together again?—the surface of the sphere has a limited area—do they do so simultaneously and after fitting together almost perfectly bounce back again, or do they slide over each other?” Perhaps lessons drawn from the progress of other sciences, of which geophysicists were doubtless ignorant, would help: “the past half century has seen enormous strides in the theory of stellar structure that have depended as far as the surface is concerned on ignoring practically everything . . . the procession of surface events associated with the sunspots, fascinatingly intriguing as these are, do not find any place in the theory”.

Nevertheless, Professor Lyttleton² recognized “that at present there is a large number of geophysicists who are inclined strongly to the view that continental drift is occurring, just as there are probably many people still that are inclined to the view that flying saucers visit this planet”. How to convince them that “these conclusions are based on entirely inadequate evidence” other than by using the same “arguments of a verbal kind” which he was condemning? Obviously what was needed was quantitative refutation of key parts of the theory!

The paper⁶ of mine, to which he refers, is largely concerned with arguing that continental drift is brought about by thermal convection in the solid mantle, made possible by a solid state creep process. The analysis of satellite orbits had revealed the presence of non-hydrostatic, low harmonics of the geoid, confirming earlier work by Jeffreys, but geodesists had attributed them to the classical view of a rigid mantle in which density anomalies had arisen, accidentally, in the early history of the Earth. I argued that this important discovery could be better explained by the density differences driving the convection currents in the mantle, and that therefore the data contained information about the pattern of laminar flow in the mantle. Until recently, geodesists ignored this theory, presumably hoping it would go away, but Professor Lyttleton² took a firmer line: “the departure from hydrostatic equilibrium was said to be due to the currents themselves. Clearly, however, there is no possibility of currents as small as 1 centimetre per year producing non-equilibrium elevations of the free surface of the Earth of the order of 0.1 km. The energies per unit mass involved in the currents is negligible, and it would require speeds of many metres per second to achieve any appropriate surface elevation even were some suitable mechanism available.” But Bernoulli's equation, appropriate in determining the height to which a fountain will play, cannot be applied to the mantle⁷, for, as I had shown⁸, the viscous term in the Navier–Stokes equation of flow in the Earth's mantle is about 10^{20} greater than the inertia and acceleration terms. In fact the distortion of the gravitational equipotential surface due to convection equals the viscous (or buoyancy driving) force per unit volume (10^{-2} dyne cm^{-3}) multiplied by the cell size (10^8 – 10^9 cm) divided by the gravitational acceleration and density, and is of the right order of magnitude.

The second criticism which he made of my paper⁶, and which he now renews in his letter¹, concerns its most speculative part. I had suggested that the break-up of the protocontinents and the present directions of movement of the continents arose from a change in the convection pattern in the Earth's mantle about 100–200 m.y. ago. I pointed to the clustering of the ages of rocks in the shield areas all over the world around three dates in the Precambrian as evidence that earlier changes in the convection pattern and earlier upheavals of the continents had taken place—a speculative idea, though

recently receiving considerable support from palaeomagnetic surveys of Precambrian rocks⁹. I finally suggested that such successive changes in the convection pattern might arise from the changing geometry of the convecting mantle by the gradual settling of iron towards the centre, suggested originally by Urey¹⁰. To illustrate the possibility that this hypothesis of a growing core might be tested by measurements of the supposed daily, monthly and annual growth increments in certain Phanerozoic fossils¹¹, I calculated the changes in the moment of inertia (and in the gravitational energy) as the core grew during the Earth's life. For the case of an iron core, these changes are the first order effects of a growing core, while the changes in the overall radius of the Earth are a second order effect, as can easily be seen by a rough calculation; thus in my work I took the radius of the Earth to be (nearly) constant. This Professor Lyttleton criticized as "manifestly invalid", claiming to have solved the problem exactly, in a paper which he submitted to the Royal Astronomical Society "On Possible Causes of Change in the Volume of the Earth" and read at the meeting of the society on January 9, 1970. The report of this meeting¹² and the paper were not seen by me until September of that year when the society decided to publish it. I then wrote a letter to *Observatory*¹³, and a paper to follow his in the *Geophysical Journal* of the society, explaining that I had made an entirely legitimate approximation ("which is a discernment that certain factors and complications do not contribute appreciably . . . a faculty to be cultivated, not a vicious propensity"—Eddington quoted by Lyttleton²). I also corrected an error in the ordinate scale of Fig. 11 (b), which should read $0-1.8 \times 10^{38}$ erg, not $0-18 \times 10^{38}$ erg. I should perhaps in my original paper have specifically said that the calculated quantities—moment of inertia and gravitational energy—of the models were accurate to about one per cent.

Professor Lyttleton's solution, using the mathematical methods already described with which he was well satisfied ("such questions require formal analysis for their safe treatment"), is given in *Observatory*¹² and in his review², and was developed fully in the paper already referred to. It is that, if the Earth had been a uniform mixture of iron and silicate at its origin and the iron had settled to form the core, its total gravitational potential energy (W) would have changed from 0.2263 to 0.2521×10^{40} erg and its moment of inertia factor (f) from 0.3789 to 0.3187 , the radius of the Earth decreasing by 43.0 km. This latter he described as "of great geophysical importance"² and commented "Whether this is enough for all mountain building is a geological question, but, if not, there remains the phase-change hypothesis". I had to point out, however, that nowhere in Professor Lyttleton's discussion was the heating of the earth by the release of about 2.6×10^{38} erg of gravitational potential energy taken into account¹³. The Earth heats up and expands, the latter process absorbing perhaps a quarter to a third of this energy. As its volume is about 10^{27} cm³ and the thermal capacity of silicates at NTP about 0.6 cal cm⁻³ °C⁻¹, its internal temperature would rise by between $2,000^\circ$ C and $3,000^\circ$ C, if no heat escaped (allowing for a possible increase by a factor of two to three in the thermal capacity towards the geocentre). The linear coefficient of thermal expansion is about 10^{-5} °C⁻¹ in the laboratory, giving an increase in the Earth's radius of 2-3%, 120 km-180 km, more than annulling Lyttleton's effect, even if the poorly known decrease of the coefficient with depth is as much as a factor of five at the geocentre (three is usually given). The values for the moment of inertia and the gravitational energy change by a similar proportion. After I had corrected the proof of my paper, the Royal Astronomical Society informed me that Professor Lyttleton had withdrawn his paper.

I now return to the incompleteness of the equation of state he uses. In the Earth and Venus the change in density with depth due to pressure greatly outweighs that due to temperature. For example, in the Earth's mantle, the density changes from 3.3 at the surface to 5.6 at the core-mantle interface, but the most the density decreases due to temperature is only 0.3.

The neglect of temperature is not too serious for many purposes except for precise calculations and the determination of second order effects. In the Moon and Mars, however, the changes of density due to pressure and temperature are comparable, and as their thermal state is almost entirely unknown Lyttleton's conclusions² concerning the non-existence of small iron cores (that is, of radii some hundreds of km) in these bodies are unsound. Analogies with Eddington's work on the internal constitutions of stars are misleading. The physics of the solid states does not lead to any simple, widely applicable equation of state like the gas laws and the chemistry of planetary interiors is complex. But solid cosmic bodies possess a memory: the key to their evolution and constitution lies in the physico-chemical record preserved at their surfaces.

Professor Lyttleton's present letter¹ alleges that in my reply¹³ to him I confused the mathematics and the physics of the problem. But unless one has a definite theory of what happens to the released gravitational energy, it is clear that the problem cannot be specified to the precision he seeks. The lack of this invalidates hundreds of calculated parameters (r_s , f , W), given to four or five significant figures, in the tables of his papers^{2,4} if we are discussing accuracies better than about one percent. It is of interest that Urey¹⁰, Birch¹⁴ and Tozer¹⁵ have also tackled, with various assumptions and approximations, the problem of the energy released per unit mass on the formation of the Earth's iron core, and obtained 670 cal g⁻¹, 600 cal g⁻¹, and 560 cal g⁻¹ respectively. I obtained 600 cal g⁻¹.

Regarding the boundary conditions of my solution, I set out completely, but clearly too briefly, what I did. The three first order, non-linear, simultaneous differential equations (see para. 2), which relate pressure p , gravitational acceleration g , and density ρ at all points in the Earth, were integrated inwards from the surface where $p=0$ and its radius and g on it taken to be the present values. The surface value of ρ_s is varied between models to represent different mixtures of iron and silicate in the mantle. But consider an entirely general model of a planet, chemically homogeneous, with a known relation between ρ $dp/d\rho$ and p applying everywhere; it would be found that, following the procedure just described (assuming a fixed external radius and arbitrary, but allowable, values of ρ and g at the surface), g and dp/dr would not vanish at the centre: this mathematical planetary model would possess a central point mass except for certain values of ρ and g at the surface. Suppose now a core, of unknown radius r_c , is inserted in the model, with a different but known $\rho-p$ relation; the point mass can be eliminated and r_c uniquely determined by the imposition of the boundary condition that $dp/dr=g=0$ at the centre. I do not see how Professor Lyttleton arrives at an "infinity of solutions" for each ρ_s unless the elementary boundary conditions at the core-mantle interface are omitted. It follows that he is incorrect in stating that "clearly the models [I] obtained have different masses to overcome the incorrect assumption of unchanging radius". Any approximation in a calculation does of course carry others with it and this work is no exception. The overall mass ratio between the silicate and iron varies slightly between each model, but mass transfer between the core and mantle is the point of the calculation. The question naturally arises whether there is a more logically satisfying procedure and I will go into this in detail elsewhere, but I think that, such is the nature of the physical problem, an approximation of the order I made will enter either early or late. Professor Lyttleton does not see this. He is also incorrect in stating that I do not explain how the timescale of the evolving core is obtained: this is given on pages 242 and 243 of ref. 6.

As Professor Lyttleton's results from his mathematics on the iron core model in his withdrawn paper still stand in the *Observatory* and his review² (Table IX) and on the phase-change core models in his other papers^{2,4}, might I suggest how a little physics can reinstate them as definitive, exact solutions of the evolving core problem? Clearly the released gravitational

potential energy must be removed as the core grows and, as I note that he has not taken up my previous suggested solution¹³ of a new cosmological principle, only heat transport remains. Tozer¹⁶, has shown that the hypothesis of thermal convection throws an entirely new light on the vexed question of the thermal history of the Earth, Moon and planets. From the well known result that creep rates depend on a Boltzmann factor, he argues that when the temperature of an initially cold planet reaches a certain critical value, convection then prevents any further appreciable change in the temperature distribution, carrying away all further heat released. But introducing "actual bodily currents into the solid region of the Earth by way of explanation of the observations" seems "premature"¹². Only conduction remains: but in bodies of planetary size the diffusion of heat by conduction takes place very slowly—typically 100 km in 10^9 y. It therefore follows that the internal temperature distribution of the evolving Earth will remain sensibly constant only if the core forms over (approximately) 10^{13} yr. Provided that this is acceptable, I am ready to admit that Professor Lyttleton has solved the problem of the growing core with satisfying mathematical rigour, superseding my approximate solution, which in spite of being "entirely valueless" seems to have put him on the path to a result of momentous cosmological importance.

Before geochemists rush in to protest that the Earth is only 4.6×10^9 yr old may I suggest that they study Professor Lyttleton's critical appraisal² of their scientific method? Their determinations are made on a minute sample of only "one hundredth of one per cent of the whole mass of the Earth . . . and, coming as it must entirely from the present surface layers, even this material may be to some extent peculiar in that it has undergone processes from which the deep interior may probably always have been screened" (two "homely parables" about paint on automobiles and soot on chimney sweeps follow). Let them not therefore add to the "welter of uninformed invalid criticism, that rolls back like the stone of Sisyphus on any theoretician who attempts to bring some order to the rag-bag of raw observations" in which, clearly, radioactive age determinations belong as well as "directions of magnetization of rocks, curious magnetic anomalies on the ocean floor, actual displacements at faultings" geologists' "multitudinous observations in the field", shock-wave velocities in rocks to say nothing of "mere correlations" such as "the close fit of the margin of the African continent into that of South America".

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Mantle Plumes, Palaeomagnetism and Polar Wandering

DUNCAN *et al.*¹ have analysed the igneous activity in the Central Volcanic province of Europe and the Thulean province of Iceland, the Faeroes and the British Isles on the assumption that these provinces represent plume traces. Assuming that plumes are fixed to the mantle and that plume traces are due to lithospheric plate motion only, Duncan *et al.* showed that the motion of the European plate over the past 50 m.y. is not in accord with palaeomagnetic data unless motion of the mantle with respect to the rotation axis (polar wandering) has occurred. Polar wandering of 23° along the 123° meridian was required since the early Tertiary.

Unless some absolute frame of reference, such as fixed mantle plumes, can be found polar wandering is an unnecessary concept in plate tectonics because the only available frame of reference is the Earth's axis of rotation^{2,3}. But, McKenzie² has shown that the concept of polar wandering could be useful if the rate of relative motion of all plates relative to the pole is very much reduced by a particular choice of polar wandering direction and velocity. In the present discussion it is relevant to consider whether or not there has been any slip of the lithosphere as a whole with respect to the Earth's axis of rotation over the past 50 m.y. This can be done by finding the vector sum of the horizontal displacements of all points on the Earth's surface with respect to the axis of rotation during this interval. An estimate of this net displacement over the past 50 m.y. can be made using palaeomagnetic data for the six major plates of Le Pichon⁴.

McKenzie² has shown that the motion of the palaeomagnetic pole P relative to a plate A can always be described by means of an angular velocity vector $\mathbf{A}\Omega_P$ passing through the equator. To allow for the different sizes of plates it is convenient to define another vector $\mathbf{A}\mathbf{M}_P$ given by

$$\mathbf{A}\mathbf{M}_P = f_A \mathbf{A}\Omega_P$$

where f_A is the fraction of the Earth's surface area occupied by plate A. The magnitude and direction of polar motion with respect to the whole lithosphere (which can be considered a form of polar wandering) is then given by the resultant angular velocity vector $\mathbf{S}$, also passing through the equator, where

$$\mathbf{S} = \sum_{n=1}^N \mathbf{n}\mathbf{M}_P$$

and N is the number of plates.

The best estimates of the positions of the palaeomagnetic pole with respect to each of the six major plates approximately 50 m.y. ago (early Tertiary) are given in Table 1. The position for the Eurasian plate is the mean of 10 poles derived from the British Tertiary igneous province ($N=10$, $k=103$, $A_{95}=5^\circ$), for the American plate it is the mean of 6 early Tertiary poles derived from cleaning ($N=6$, $k=23$, $A_{95}=14^\circ$), for the African plate it is given by the result from the Ethiopian flood basalts⁵ and for the Pacific plate by the data from the California seamounts⁶. The position for the Indian plate is given by the result from the Barrington Volcano in eastern Australia⁷, but because there are no results from the lower Tertiary of

Table 1 Magnitude and Direction of Polar Motion with respect to the Six Major Plates over the past 50 m.y.

Plate	Pole (~50 m.y.)	f	Ω (10^{-2} deg Myr $^{-1}$)	M (10^{-2} deg Myr $^{-1}$)	Pole of rotation
Eurasian	75 N, 161 E	0.12	30	3.60	0 N, 71 E
American	85 N, 197 E	0.20	10	2.00	0 N, 107 E
African	81 N, 168 E	0.16	18	2.88	0 N, 78 E
Pacific	71 N, 354 E	0.22	38	8.36	0 N, 264 E
Indian	70 N, 306 E	0.14	40	5.60	0 N, 216 E
Antarctic	80 N, 175 E	0.11	20	2.20	0 N, 85 E

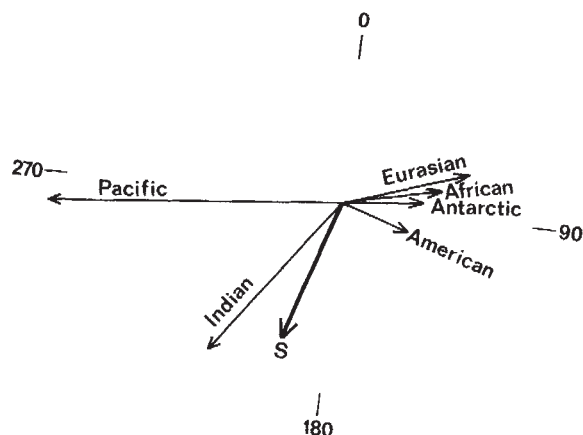


Fig. 1 The vectors M describing the polar motion with respect to the six principal plates covering the Earth's surface. The resultant S is given by the thicker line. All the vectors lie in the equatorial plane.

Antarctica, the position for the Antarctic plate is found by subtracting the relative motion between Antarctica and Australia (from seafloor spreading) from the Australian Tertiary data⁸. The six principal plates now occupy 95% of the Earth's surface so that the motions of the minor plates may be ignored, although some uncertainty arises from the assumption that the six plates have maintained their present size over the past 50 m.y. In estimating Ω for each plate, I assumed that the pole has followed the most direct path along a meridian to its present position.

The magnitudes and poles of rotation of the six vectors M_p calculated in Table 1 are illustrated in Fig. 1. The resultant S has pole of rotation at 0°N, 198°E and magnitude 2.1° corresponding to motion of the pole with respect to the whole lithosphere of 2.1° along the 108° meridian. This is certainly not significantly different from zero. Even if the poles of rotation for the six plates all coincide, the maximum would be 12.3° . The analysis shows that the conclusions drawn by McElhinny and Wellman⁸ about polar wandering since the Eocene are incorrect because they considered only half of the Earth's surface. The integrated effect of motions over the whole surface should be considered. I conclude that there has been no significant motion of the lithosphere as a whole with respect to the Earth's axis of rotation over the past 50 m.y. On the other hand, the calculation of Duncan *et al.*¹ suggests a 23° movement of the mantle with respect to the rotation axis, at least an order of magnitude greater than any lithospheric motion. If I accept the plume calculation I am forced to the surprising conclusion that the lithosphere remains relatively fixed with respect to the rotation axis, but that the mantle inside moves with respect to the same axis. The alternative conclusion is that the underlying assumption made by Duncan *et al.*, that plumes are fixed relative to the mantle, is incorrect.

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Polymers of Improved Modulus by High Pressure Polymerization

SOME highly orientated organic fibres¹ are now available with a Young's modulus as high as 130 GN m^{-2} , but most rigid polymers have values between 2 and 4 GN m^{-2} . Even in cross-linked materials, such as phenol formaldehyde resin, it is difficult to achieve values in excess of 7 GN m^{-2} . The reasons for this are to be found in the difficulty in random three dimensional structures of building up a large enough number of bonds per unit volume to have a significant effect upon the properties. It has been suggested (ref. 2 and R. N. Haward, private communication) that cross-linking of organic polymers at high pressures might raise the number of bonds sufficiently to have an appreciable and permanent effect on density, modulus and other physical properties; we have demonstrated that this is so using *p*-divinylbenzene as a model difunctional monomer.

Pure *p*-divinylbenzene³ in thin walled gold capsules was polymerized by heating to $200\text{--}250^\circ \text{C}$ at the desired pressure in a piston-cylinder apparatus⁴. In some experiments *p*-methoxyphenol (100 p.p.m.) was added as inhibitor to ensure that polymerization did not begin before the full polymerization pressure was reached. The density of the product was measured in a density column, the infrared spectrum as a KBr disk and Young's, shear and bulk moduli by an ultrasonic pulse technique⁵ at 5 MHz.

Fig. 1 shows the dependence of the density of poly(*p*-divinylbenzene) upon the pressure of polymerization in experiments with inhibited *p*-divinylbenzene. The density of material polymerized at a few atmospheres pressure is about $1.05 \text{ (g ml}^{-1} \text{ at } 23^\circ \text{C)}$ which may be taken to be close to the density of *p*-vinyl polystyrene, which would be expected to be below that of polystyrene (~ 1.06). The polymer produced at the highest pressures used in these experiments ($\sim 2 \text{ GN m}^{-2}$) has a

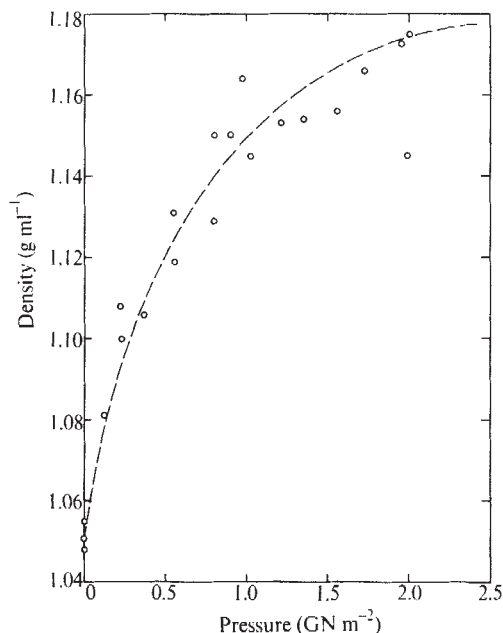


Fig. 1 Variation of density of poly(*p*-divinylbenzene) with pressure of polymerization.

density of ~ 1.175 . It is interesting that styrene containing 20% divinylbenzene gave at 1.25 GN m^{-2} a polymer of density 1.088 gm ml^{-1} compared with ~ 1.155 for *p*-divinylbenzene at the same pressure. This demonstrates the effect of reducing the maximum attainable number of cross-links in an otherwise fairly similar system.

Fig. 2 shows the relationship between density and residual vinyl group for all polymerizations. The concentration of residual vinyl groups was estimated from the intensity of the bands at 901 and 837 cm^{-1} in the infrared spectra of the products using the relationship

$$[\text{vinyl groups}] \propto \log_{10} T_a^{-1} / \log_{10} T_b^{-1}$$

where T is the transmittance at the specified frequency. If it is assumed that polymer of density 1.05 g ml^{-1} is substantially *p*-vinyl polystyrene then about 36% of the vinyl group still present at this stage—about 18% of that in the monomer—remains as free vinyl group or end group unsaturation in the densest of these materials. Nevertheless the effect upon the moduli is marked. Fig. 3 shows Young's modulus plotted

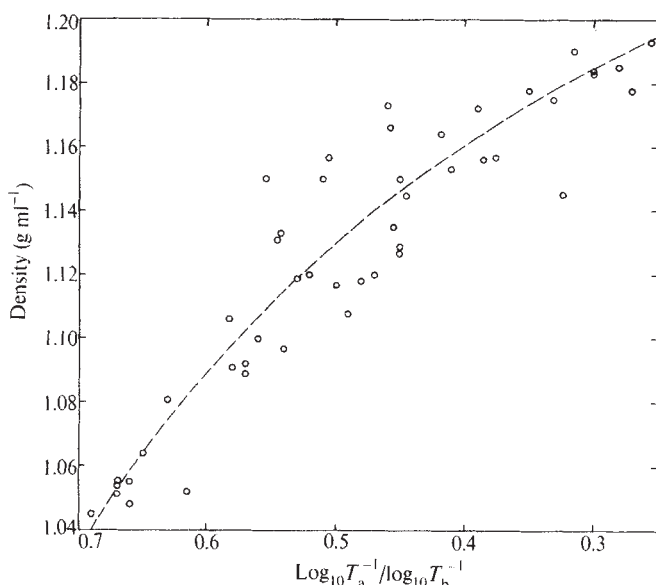


Fig. 2 Plot of density of poly(*p*-divinylbenzene) against $\log_{10} T_{901 \text{ cm}^{-1}}^{-1} / \log_{10} T_{837 \text{ cm}^{-1}}^{-1}$ ($\propto$ [residual vinyl group]).

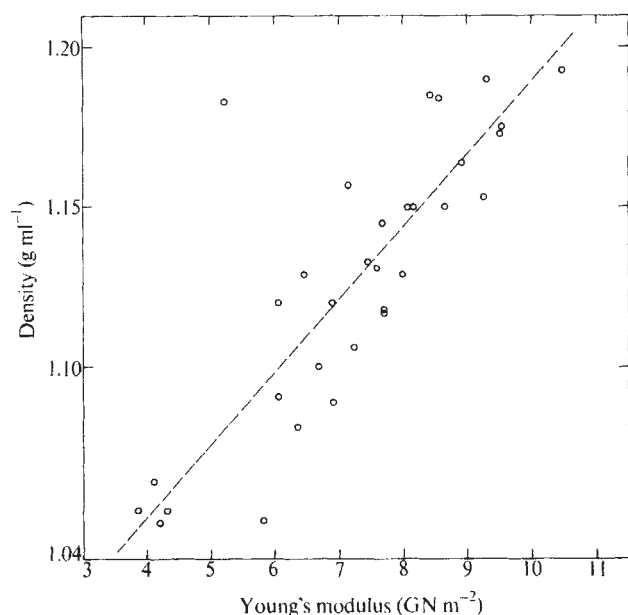


Fig. 3 Plot of density against Young's modulus.

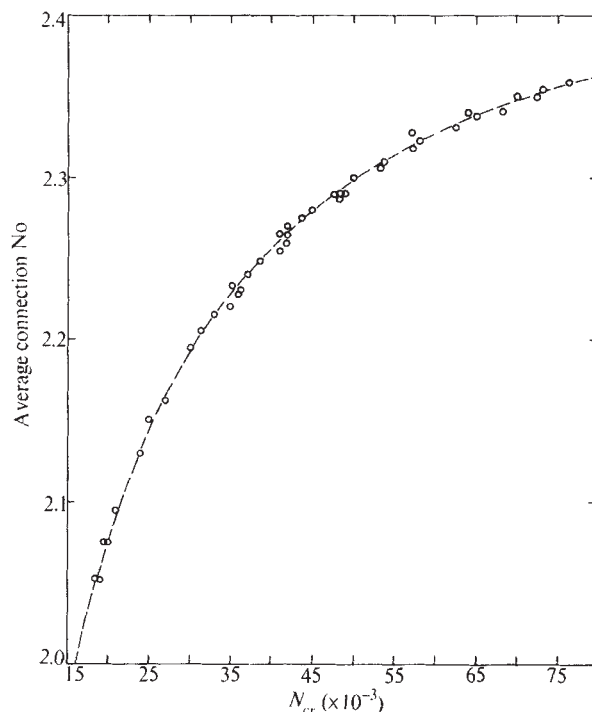


Fig. 4 Plot of average connexion number of *p*-divinylbenzene polymers against N_{cr} .

against density and the shear and bulk moduli show a similar variation: a substantial increase is obtained in all cases.

The concept of connexion number⁶, which provides a means of describing regular covalent networks, has been adapted to random polymer networks⁷. The maximum average connexion number of poly(*p*-divinylbenzene) is 2.4 while that of the densest of our products is about 2.36. More recently a new parameter N_{cr} has been introduced⁷; it is the relative number of covalent network bonds per unit volume in a polymer. Fig. 4 shows a plot of connexion number against N_{cr} for all polymerizations.

The *p*-divinylbenzene system, though a convenient model, is unsuitable for the preparation of practically useful materials. It is unlikely for steric reasons that any appreciable further increase in average connexion number could be achieved even at much greater pressures and, in addition, the products are extremely brittle. However, a few experiments with mixtures of *p*-divinylbenzene and *p,p'*-divinyldiphenyl ether have shown the same trend with the production of less brittle materials and it seems likely that with careful choice of polyfunctional monomer the theoretically possible average connexion number might be more closely approached and that practically useful materials might be obtained.

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Icelandic Geothermal Activity and the Mercury of the Greenland Icecap

A RECENT controversy over mercury found in Greenland ice cores¹⁻³ has served to underscore the need for more information about natural sources and modes of dissemination of the element.

Following the demonstration of high levels of atmospheric mercury in Hawaiian thermal areas⁴, similar aerometric studies were conducted at a number of sites in Iceland during June and July 1972. Field air sampling procedures and analyses by flameless atomic absorption were carried out as described in the Hawaiian study.

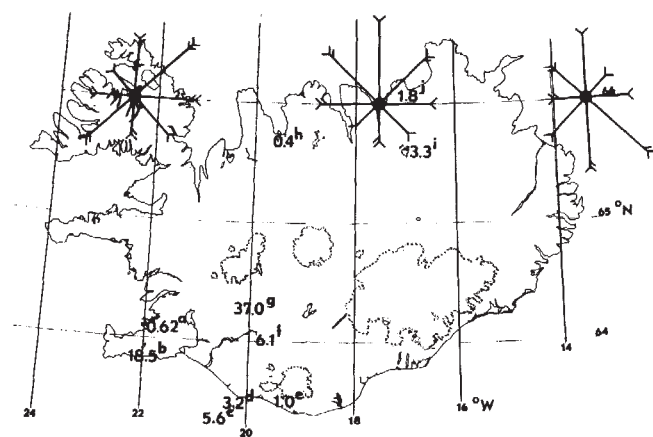


Fig. 1 Aerometry of mercury in Iceland. Located on the outline map are the atmospheric mercury levels (in $\mu\text{g m}^{-3}$) in June and July 1972, and representative wind vector ("windrose") patterns for June 1972. Mercury sampling sites were: a, Reykjavik; b, Krýsuvik; c, Surtsey; d, Heimaey; e, Solheimajökull; f, Hekla; g, Geysir; h, Varmahlid; i, Myvatn; j, Húsavík.

Although atmospheric mercury levels vary greatly according to site, samples from several widely separated locations (Fig. 1) yielded Hg concentrations well above the range commonly cited for unpolluted air⁵, $0.001\text{--}0.010 \mu\text{g m}^{-3}$. The general reliability of field measurements is indicated by samples taken at the Krýsuvik thermal area where duplicate analyses on each of four separate sample traps yielded a mean value of $11.3 \mu\text{g m}^{-3}$ and a standard error of only $\pm 1.8 \mu\text{g m}^{-3}$. In the Westmann Islands (including Surtsey), five sites yielded a mean Hg level of $2.4 \pm 0.8 \mu\text{g m}^{-3}$.

Atmospheric mercury may be introduced in part by degassing of fluid magmas, but release from fine ash could also serve as a vehicle. Thus, an acid condensate from Surtsey yielded on analysis 350 p.p.b. Hg and tephra fines collected in the first two hours of the 1970 Hekla eruption contained 375 p.p.b. of readily water-soluble Hg.

Long range transport of volcanic ejecta eastward from Iceland is well established. Thorarinsson^{6,7} notes that in 1947, tephra was deposited in Finland, some 3,000 km distant, two days after Hekla began its great eruption; and that the vast gas emission accompanying the Lakagigar eruption of June 1783 caused a blue haze which spread for months over all of Iceland, most of Europe and proximal parts of Africa and Asia. Although these episodes show the feasibility of atmospheric transport, advection from Greenland to Iceland would be favoured by prevailing westerly winds. Examination of North Atlantic wind vector patterns⁸ (Fig. 1) show that the winds (in June, for example) may blow as much as 30–50% of the time from east to west across Iceland toward Greenland. Furthermore, North Atlantic gales, which are commonly associated

with eastward moving cyclones, sustain a westward circulation in their northern sectors, and provide additional means for conveying effluents from Iceland to Greenland⁹.

The presence of substantial Hg levels in deep North Atlantic sediments located S, SW, and W of Iceland¹⁰ may also reflect long term transport and fallout of Icelandic mercury in nearby waters⁹.

Whatever may be the significance of time-variable mercury levels, they cannot be viewed as evidence for human input when it is recalled that from the mid-17th century to the present, Iceland has recorded nearly 50 volcanic eruptions including some of the greatest in the post-glacial history of the Earth.

We thank Dr Gudmundur E. Sigvaldason and the Division of Geosciences at the University of Iceland for generous laboratory facilities and cooperation in field collection and sampling. This work was supported in part by the National Aeronautics and Space Administration, the Cottrell Foundation of the Research Corporation, the University of Hawaii Foundation, and the University of Hawaii Environmental Center.

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Distribution of Dissolved Mercury in the Irish Sea

PRESTON *et al.*¹ have recently examined the distribution of a number of heavy metals in British coastal waters. They have found that the average concentrations of these elements in waters and marine organisms were significantly higher in samples from the eastern Irish Sea than in those from other areas. Fish and shellfish from this region (particularly the Mersey Estuary and Lune Deep) have been observed to have the highest average contents of mercury of any from around the British Isles². No data are available for the concentration of the element in the seawater of the Irish Sea, nor is anything known about whether significant mercury pollution arises from liquid wastes discharged into the sea from the populous, highly industrialized areas of Lancashire. Trade effluents from

the many chemical and industrial plants (including three chlorine-alkali works) would probably be the principal sources of mercury, but the input from sewage, which may contain a significant concentration of mercury (2–150 p.p.m. on the dry weight)^{3,4}, would also be important, as the total daily discharge of (mainly untreated) sewage to the area amounts to about $0.7 \times 10^6 \text{ m}^3$ (ref. 3). We describe here the distribution pattern of dissolved mercury in the Irish Sea, and have demonstrated that significant pollution occurs in the eastern coastal strip.

Six cruises were made between July 1971 and September 1972 to collect surface waters in an area east of a line between St. Bees Head and Anglesey. In addition, a surface water sampling programme was carried out in September and October 1972 covering the remainder of the Irish Sea. Immediately after collection, the sample (2.5 l.) was filtered through a treated membrane filter (pore size $0.45 \mu\text{m}$) and the first 500 ml. of filtrate was rejected. The remainder of the filtrate was acidified to 0.2 M with sulphuric acid and transferred to silicone-coated glass bottles for transport to the laboratory. Tests showed that <20% of the dissolved mercury was lost during the filtration, and that the acidified samples could be stored safely for several weeks. A combination of dithizone extraction and cold vapour atomic absorption spectrophotometry⁵ was used for the analysis of the samples. Organic forms of mercury such as methyl mercury as well as inorganic mercury are determined by this technique⁶.

The survey carried out in September–October 1972, showed (Fig. 1) that excluding the coastal strip about 40 km wide extending northwards from Anglesey, the dissolved mercury content of the Irish Sea lay almost entirely in the range 25–50 (average 32) ng l^{-1} except for the central area in which it ranged from 10–24 (average 18) ng l^{-1} . The overall range of values is similar to that found for the English Channel⁷, the northern North Sea⁸, and the NE Atlantic⁹.

The six surveys of the eastern coastal strip showed a consistent picture (Fig. 2). The general levels of dissolved mercury in the inshore waters are similar to those found in the westerly regions examined in the cruise in September–October. These concentrations probably represent the equilibrium values for the distribution of mercury between water and suspended matter. Offshore, there are two areas in which the dissolved

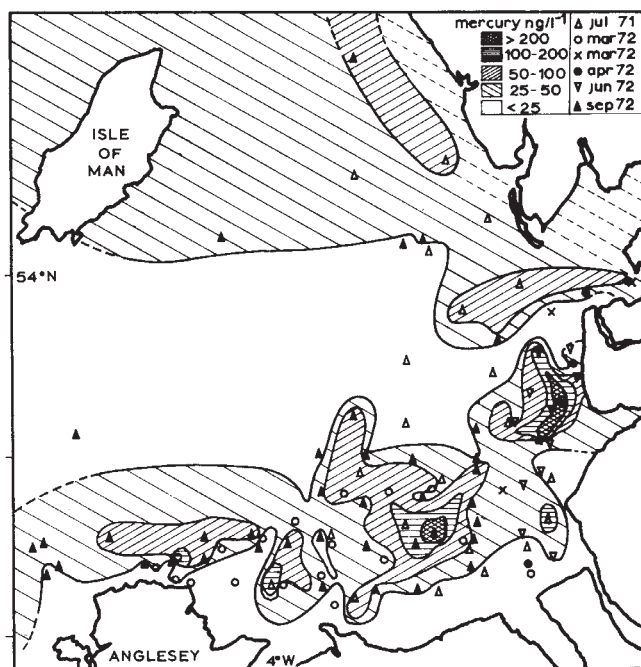


Fig. 2 Distribution of dissolved mercury in the eastern coastal area of the Irish Sea (as ng l^{-1}).

mercury concentration exceeds 200 ng l^{-1} . One of these is in the vicinity of the North West Light, an area which is used for the dumping of sewage sludge from the Manchester conurbation¹⁰.

Dried sludges are known to contain up to 150 p.p.m. of mercury and, in fact, concentrations of particulate mercury of up to $\sim 900 \text{ ng l}^{-1}$ were observed in the area. We tentatively suggest that the observed high concentrations of dissolved mercury found at this station were liberated during bacterial decomposition of the sludge. The dissolved mercury content is also relatively high in an elongated patch to the west of the Ribble Estuary. The highest concentration observed in this region was 443 ng l^{-1} , but the source of the pollution in this area is uncertain. In view of the very low levels of dissolved mercury to the north, it seems very unlikely that it originates from effluents from the ICI chlorine-alkali plant on the River Wyre. It is probably significant that these two areas lie within that part of the Irish Sea yielding fish which have an average mercury content (0.53 p.p.m. dry weight) more than double those from the rest of this sea (average 0.21 p.p.m.)².

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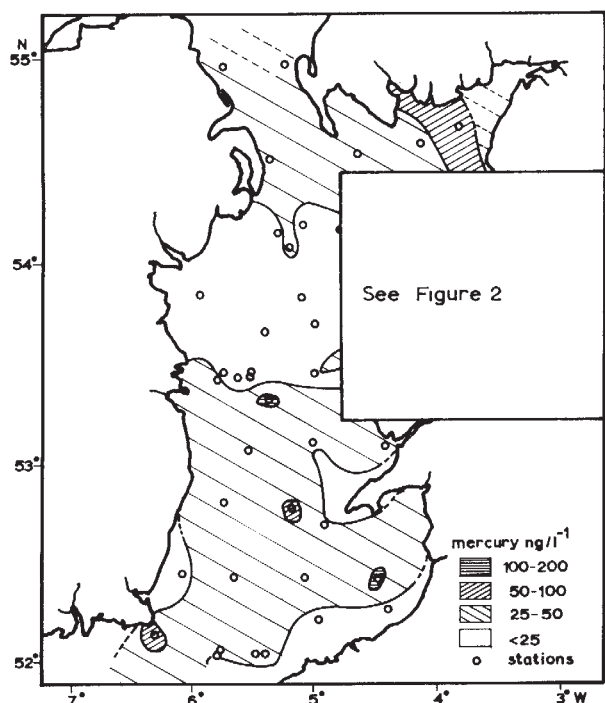


Fig. 1 Distribution of dissolved mercury in the Irish Sea in September–October 1972 (as ng l^{-1}).

BIOLOGICAL SCIENCES

Localization of Paternal DNA in Interphase Nuclei of Mouse Eggs during Early Cleavage

TRANSFER of labelled paternal genome has been demonstrated during fertilization in mammals^{4,5,14,15}. The ¹⁴C adenine used, however, did not permit precise localization of parental material within the nuclei of the dividing egg. These and other observations using tritiated thymidine (³H-TdR)⁸ were made on histological sections of eggs, limiting the precision of the localization of the entire paternal DNA complement in egg chromatin. Intense labelling of the germinal cell line in male mice has been described, using ³H-TdR^{7,11}, and we have combined this by using the air-drying method of Tarkowski¹⁶ (which gives good preparations of complete egg nuclei) to localize the paternal DNA complement.

Male mice (inbred CBA T6/T6 strain) were injected intratesticularly with ³H-TdR¹¹. They were mated with females, superovulated with gonadotrophins⁶, on the day of peak-production of labelled spermatozoa, as shown by 50% labelled epididymal sperm, or sperm collected by vaginal washings shortly *post-coitum*¹³. This was found to be 32 to 34 day^{1,7,9}. The time of mating was calculated from the time of appearance of a vaginal plug. Females were killed from 36 to 60 h post-mating, corresponding to two to eight cells in the cleavage stages of the egg³, and were given colchicine 1.5 to 2 h before death¹⁶. Oviducts were dissected and eggs collected with a micropipette under a dissecting microscope by flushing with Hanks medium. After 6–8 min in hypotonic citrate, eggs were fixed with one drop of acetic alcohol (1 : 3), air-dried and treated with a further two drops of fixative at the beginning of spreading¹⁶. Autoradiographs (ARG) ('Kodak NTB-2' or 'Ilford L-4' dipping emulsion) were exposed for 60 day and stained through the film with 2% toluidine blue.

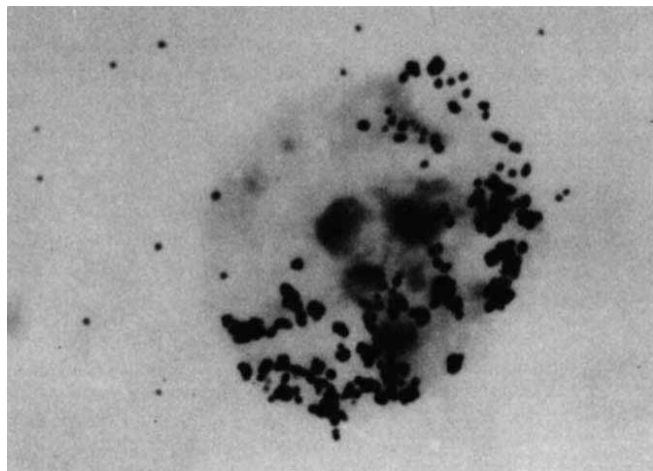


Fig. 1 Patchy and polar localization of ³H-TdR grains in a nucleus from a 4 cells stage egg fertilized with labelled sperm.

When mating occurred at the peak of radioactive sperm production, approximately 30 to 50% of labelled eggs were recovered from a given female. ARG's of eggs fixed at the pronuclei stage revealed labelling of only one pronucleus, usually the larger one. The grains were randomly distributed over the whole nuclear area.

At later stages ARG grains were not randomly distributed and in more than 90% distribution was patchy (Fig. 1). They did not appear to be preferentially localized over any nuclear structure. In almost all labelled nuclei, grains were localized

preferentially, the remaining nuclear area being devoid of label. Polar localization of the labelled zone was apparent in 147 out of 368 labelled nuclei, ranging from cleavage stages of two to eight cells. In these nuclei, we evaluated the relative grain proportion of the total nuclear surface. In 30% of nuclei the grains extended over half of the nucleus, in 64% the zone was relatively smaller, and in 6% markedly larger than half the surface. The zone in the remaining two thirds of the labelled nuclei, with no polar localization was more difficult to locate exactly, although label was non-random.

The 220 nuclei were arbitrarily classified in three categories; (a) grain distribution in a crescent-like zone at the nuclear periphery (19%); (b) a belt-type localization in the central part of the nuclear surface (63%); (c) any other type of localization (18%, 1% completely labelled).

We suggest that there is segregation of the chromatin according to parental origin in interphase nuclei of cleaving fertilized eggs. This was particularly apparent in eggs of two to five nuclei, but impossible to assess in later stages because of low intensity of labelling (due to dilution of ³H-TdR) and the small number of eggs.

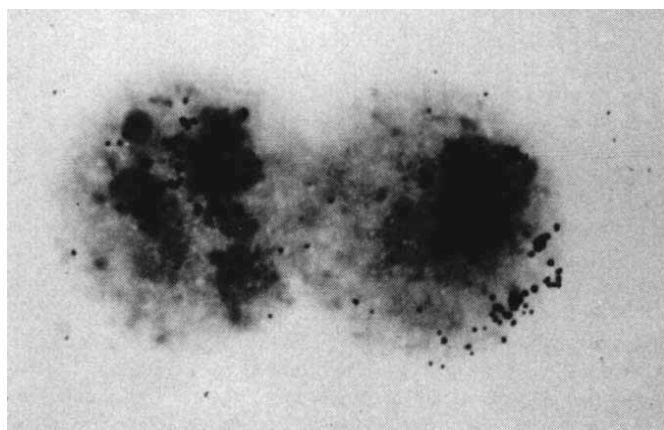


Fig. 2 Two nuclei from a 4 cells stage with different orientation polar localization.

We believe these results are not autoradiographic artefacts because: (1) No localized distribution of grains was seen in controls, namely pronuclei stages or fertilized eggs incubated *in vitro* with ³H-TdR during cleavage¹⁰, (2) orientation of labelled poles was completely different in each nucleus (Fig. 2), eliminating the error of uneven coating with ARG emulsion. We did not analyse the relative position of labelled polar zones of different nuclei in the same egg, however, as phase-contrast microscopy showed distortion of the cellular orientation during fixation.

Radiobiological artefacts cannot be completely eliminated. Lethal mutations were induced by ³H-TdR labelling of sperm¹, related to the degree of labelling. The dominant lethals were only recorded on the thirteenth day of gestation, however, and rates of fertilization and development of the zygote during early stages following *in vitro* fertilization was almost the same for normal and ³H-TdR-labelled sperm⁹.

There are two indirect cytological arguments from our work showing there are no major radiobiological lesions of the organization of the egg nuclear chromatin ³H-TdR due to: first, the mean number of ARG grains lying over all nuclei of a given zygote has been determined and compared to the stage of cleavage (from two to six cells). This mean total of 120 grains per egg (range 18 to 411), is not significantly different in any cleavage stage, nor does it diminish with advancing cleavage. We have not been able to analyse the numbers of grains between various nuclei of the same egg with relation to

the segregation of labelled material among daughter-cells because of insufficient data. Observed distributions, however, were compatible with an equal segregation of the labelled material, at least during the first division of the zygote. The patterns at later stages can also be explained, considering asynchrony in the divisions of their nuclei; second, in all the 24 labelled metaphases from eggs containing two to seven nuclei, ^3H -TdR was localized in a half or less of the chromosome complement. An exact evaluation of the distribution of labelled chromosomes has not been made, but in relative terms, metaphases at the 2 cell-stage tended to approach a Mendelian 1/1 distribution of labelled and unlabelled chromosomes.

We cannot at present make definite conclusion from these data, especially as the degree of labelling of a fertilizing sperm cannot be precisely determined. Some of them were exposed to ^3H -TdR only during the late part of the last period of spermatogonial DNA synthesis and although sperm production occurs in waves, a marked mixing in the epididymis is known to occur¹². The partially-labelled sperms may contribute to the patchy distribution of grains, in their heterochromatic component². Embryologically a topographical separation of paternally-derived nuclear chromatin in early diploid mouse eggs is reasonable. Molecular and genetic implications are that the region of the interface between the two parts leaves ample room for any grouping of allele gene pairs.

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Electron Microscopy of Negatively Stained tRNA

SEVERAL techniques have been used to determine the three-dimensional conformation of transfer ribonucleic acid (tRNA), including small angle X-ray scattering¹, electron microscopy²⁻⁵, among other physical⁶, chemical and biological methods⁷. Here we report some observations of certain "superstructures" of negatively stained tRNA using electron microscopy.

Transfer RNA samples were from *Escherichia coli* K12 tRNA (General Biochemicals Inc., Ohio) and of yeast phenylalanine tRNA (Boehringer, Mannheim, California). The *E. coli* K12 tRNA was specified as having 96% RNA, 2.2% protein,

<1% DNA, no RNAase activity, and an absorbancy of 2.0 at 260 m μ in a 0.01% solution. We used the 2% aqueous ammonium molybdate (Fisher Scientific Company, maximum impurities 0.015%) adjusted to pH 7.4 or 8.3 with 10 M ammonium hydroxide as a negative stain. Support films of 'Formvar' were thinly coated with carbon in an 'Edwards E12E' vacuum evaporator, and mounted on copper grids. In these experiments 2 mg of tRNA were dissolved in 1 ml. of negative stain and sprayed onto the support film with a glass atomizer to give droplets 5–10 μm in diameter. Some preparations were sonicated for 1 h in a 'Ladd Ultrasonicator' before spraying. All preparations were observed in a 'Philips EM300' electron microscope operated at 80 kV. Photographs were taken on 'Ilford EM5' plates and developed in 'Ilford 1D2'.

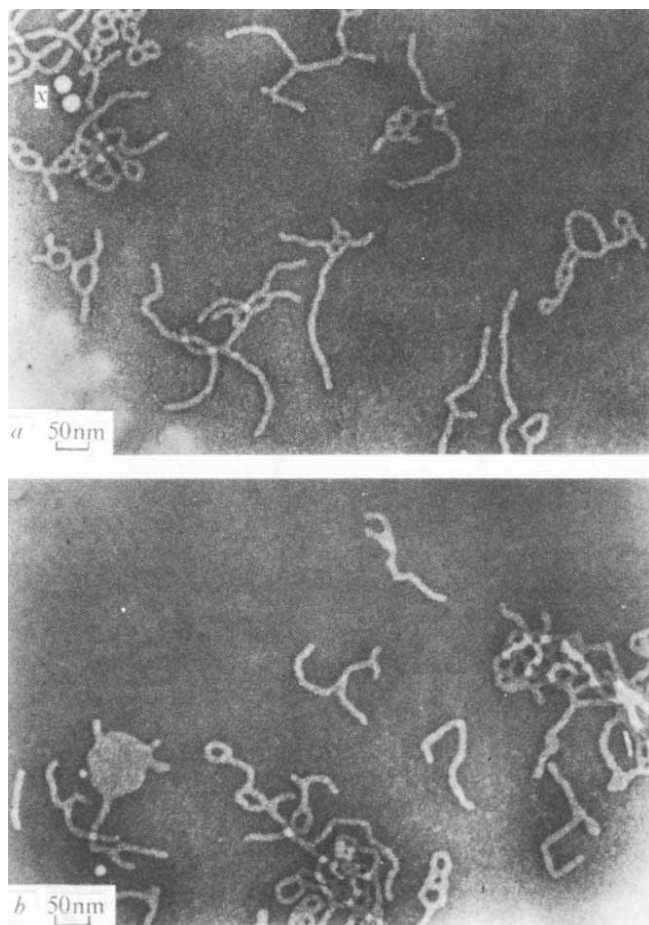


Fig. 1 *a*, tRNA negatively stained with 2% ammonium molybdate at pH 7.4, showing long branching "worm-like" structures 2 to 13 nm thick with two solid circular areas (x). *b*, As *a*, with pH 8.3. The thickness of the structures is more variable and one extensive sheet is seen.

In unsonicated preparations of *E. coli* tRNA at both pHs, long "worm-like" structures up to 0.5 μm in length were seen abundantly in most droplets of negative stain. These structures were not seen in any droplets of negative stain alone, in negatively stained yeast phenylalanine tRNA, or in sonicated preparations of negative stained *E. coli* tRNA; or were they found in *E. coli* tRNA samples which had been incubated in ribonuclease (5 $\times$ cryst., bovine pancreas, A grade, Calbiochem) for 48 h at 37° C before negative staining.

At pH 7.4 (Fig. 1*a*) these structures were seen to be of quite constant thickness (12 to 13 nm). They were usually curved,

with a minimum inner radius of curvature of 4 nm and branched frequently; always into two, and usually symmetrically at 120°. Often these branches formed closed loops. Occasionally solid circular areas about 25 nm in diameter were seen whose density resembled areas where two of the "worm-like" structures overlapped.

At pH 8.3 (Fig. 1b) these structures demonstrated some striking differences. They were more varied in thickness, occasionally spreading out into sheet-like areas of varied diameter, and closed loops were more frequent. All of these structures, as well as the thinner areas of negative stain, showed a mottled density pattern which was different from the 1.0–1.5 nm granularity of both the carbon-Formvar support film and the negative stain without tRNA.

The presence of these structures in these preparations indicates extensive aggregation of tRNA molecules in a specific manner. This aggregation could involve differences in the primary structure of the tRNA molecules, as the structures did not occur when only one species (phenylalanine tRNA) was present. The forces holding the aggregates together are not strong, because sonication disrupted the structures. To form chains or sheets of molecules, more than one site of interaction must exist on each molecule.

The two sites of chemical bonds in the cloverleaf model⁸ of the tRNA molecule which would satisfy these criteria are: *a*, hydrogen bonding between looped regions of adjacent molecules as demonstrated by Eisinger⁹; *b*, base pairing between 3' and 5' ends of partially separated stems of adjacent molecules, as suggested by Fröholm and Olsen^{2–4}. It is likely that both of these types of interaction exist, as well as some interaction with the negative stain itself.

The worm-like structures are unlikely to be cylinders or rods, as their edges appear too sharp, but they could be flat sheets or flattened cylinders. In this case the molecules are arranged about the surface of the structures as this would allow formation of extensive sheets at the higher pH used here^{2,3}. If, however, the structures are rods or cylinders, the molecules can be arranged along the axis of the rod. Dimers of tRNA have been described with a length of 10.9 nm⁵, which is in agreement with the observed 12–13 nm thickness of our preparations. The dimers could be in a rosette arrangement along the rod axis.

The mottle fine structure of these rods or sheets, as well as of the thinner areas of negative stain, is a ring of 3–4 nm, but no definite arrangement of these could be determined. We are now studying this fine structure in greater detail.

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Preliminary X-ray Analysis of the Crystalline Complex between Polypeptide Chain Elongation Factor, Tu, and GDP

DURING protein synthesis in bacteria, aminoacyl tRNA is transported to the ribosome complexed with a protein elongation factor Tu (EF-Tu) and GTP^{1–3}. In the ribosome the aminoacyl tRNA is positioned onto its appropriate codon, GTP is hydrolysed, and EF-Tu is released as a stable complex with GDP. The EF-Tu·GDP complex is then reconverted to EF-Tu·GTP by means of another soluble protein, EF-Ts. Transfer factors analogous to EF-Tu but with different physical properties have also been isolated from eucaryotic systems. The EF-Tu·GDP complex has been purified to homogeneity from *Escherichia coli*^{4,5}. The protein factor has a molecular weight around 40,000 and is composed of a single polypeptide chain. It forms a stable 1:1 complex with GDP which has a dissociation constant of 3×10^{-9} M⁴. In the purification of EF-Tu from *E. coli*, needle shaped microcrystals have been reported^{4,5} on addition of ammonium sulphate; these crystals were too small for X-ray diffraction analysis, however. Here we report the preparation of large crystals of an EF-Tu·GDP complex and describe their preliminary analysis by X-ray diffraction.

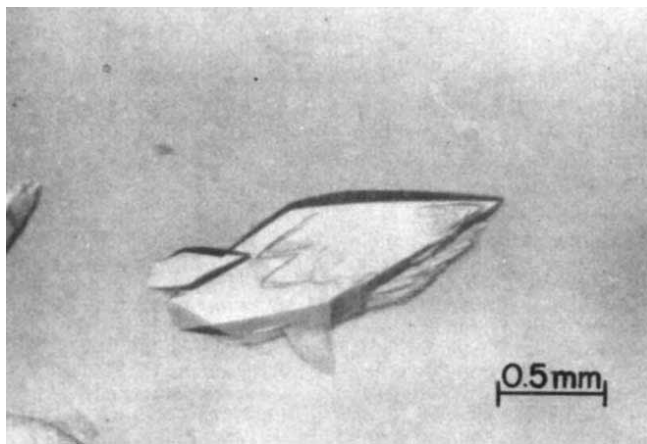


Fig. 1 Microphotograph of crystals of Tu·GDP complex. Crystals are found with both smooth and irregular faces.

We have prepared purified EF-Tu·GDP by methods described previously⁴. Larger crystals were obtained by using a method of vapour phase equilibration similar to that used for tRNA crystals⁶. A solution was prepared containing 14 mg ml⁻¹ EF-Tu·GDP, 10 mM Tris·SO₄ (pH 8.0), 0.5 mM dithiothreitol, 0.5 mM MgSO₄, 1 mM MgCl₂, 0.05 mM EDTA and 0.01 mM excess GDP. 40 µl. aliquots of this solution were put into the depressions of a 'Pyrex' spot plate which was then placed on a platform in a plastic box. A reservoir of 25 ml. of 2% isopropanol was placed into the bottom of the plastic box which was then sealed and stored at 4° C. Crystals began to appear after approximately one month. This was associated with a vapour phase equilibration of the isopropanol as well as some evaporation of the crystallizing solution. Crystals have also been obtained with up to 10 mM MgCl₂ or with a reservoir containing 2-methyl-2,4-pentanediol rather than isopropanol. These crystals appear with either the morphology shown in Fig. 1 or as tetragonal bipyramids.

The crystals were mounted in thin-walled glass capillaries together with a droplet of mother liquor for X-ray diffraction analysis. Twelve degree precession photographs with a crystal to film distance of 100 mm were taken for approximately 48 h

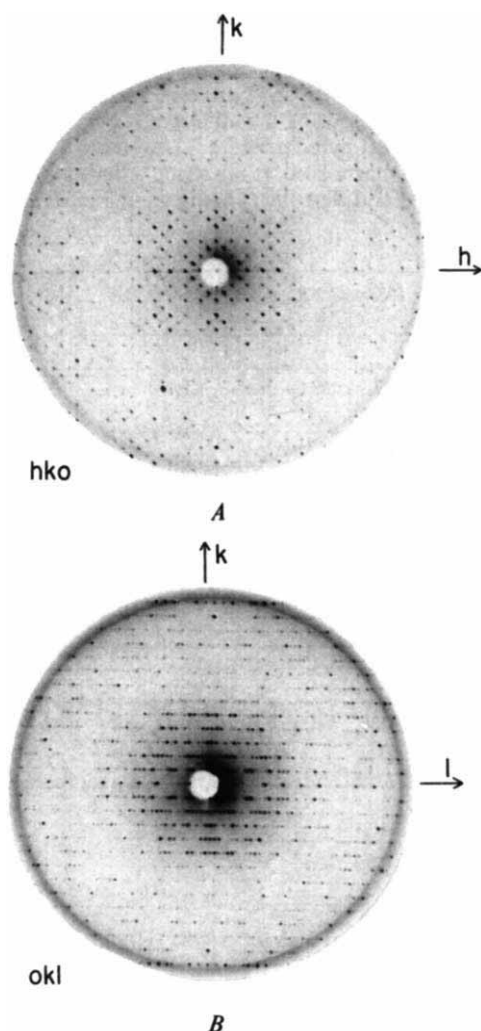


Fig. 2 Precession photographs of Tu-GDP crystals. The precession angle was 12° and the exposure near 50 h. The $hk0$ (A) and okl (B) projections are shown. The *pseudo* fourfold axis is perpendicular to the $hk0$ projection.

at 8° C using $\text{CuK}\alpha$ radiation from an 'Elliot' rotating anode source.

Two projections are shown in Fig. 2. The cell is *c*-centred, orthorhombic, and has dimensions $a=98.2$ Å, $b=100.1$ Å, $c=160.6$ Å. The space group is $C222_1$. There is a full representation of diffraction intensity out to at least 2.8 Å, making them quite suitable for detailed X-ray analysis. The crystals are stable in the X-ray beam for around one week.

The high *pseudosymmetry* is readily apparent from inspection of the $hk0$ and okl projections (Fig. 2). Examining the 001 axis, only every fourth reflexion appears, implying the presence of a fourfold screw axis. In the limit of resolution of these photographs there is no sign of a reflexion other than $1=4n$ appearing. However, the $hk0$ projection does not have true fourfold symmetry. The *pseudosymmetry* corresponds to the tetragonal space group of $P4_12_12$ with cell dimensions $a=b=70.2$ Å, $c=160.6$ Å, $\gamma=91^\circ 12'$ and a volume of one half that of the true orthorhombic unit cell. The density of the crystal measured in a density gradient is 1.20 g ml. $^{-1}$. Assuming that EF-Tu-GDP has the partial specific volume of 0.74 g ml. $^{-1}$, which is an average value for most proteins, and the density of the mother liquor to be 1.02 g cm. $^{-3}$, the protein occupies 55% of the unit cell volume. There are two molecules per asymmetric unit and these molecules are related by a *pseudo* fourfold screw axis along the *c* axis.

The protein, EF-Tu, is of interest because it is capable of interacting with guanosine nucleotides, all aminoacyl tRNA's with the exception of met-tRNA f , and with EF-Ts as well as some portion of the ribosome-mRNA complex. Structural studies of these complexes by X-ray diffraction may reveal details of the protein-nucleotide interactions and may also illuminate the currently unknown mode of action of the protein. We are trying to obtain suitable isomorphous derivatives of these crystals, and crystals of this protein complexed to aminoacyl-tRNA.

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The Amino-acid Sequence of Cytochrome c from *Euglena gracilis*

THE large number of mitochondrial cytochrome c sequences known and the elucidation of the tertiary structure of the horse heart protein by Dickerson's group¹ provide a good opportunity for studying the evolution of a molecule and for correlating structure with function. Dickerson has shown that relating sequence data to the tertiary structure can generate hypotheses about functional regions that are evolutionarily conserved. There is no general agreement, however, as to whether the differences in sequence from different species are adaptive or neutral and this is an aspect of the current selectionist versus neutralist argument².

A few protozoan cytochromes c have been purified³⁻⁷. From an evolutionary point of view, sequences of proteins from these organisms may be interesting, as they are considered the most ancient eukaryotic group and their proteins may provide homology links with prokaryotes. Such links could suggest a history for the organelle in which the protein is found. (The interpretation of this kind of evidence may not be simple: cytochrome c, for example, is found in the mitochondrion but in present-day eukaryotes is thought to be coded for in the nuclear genome⁸.) The phylogenetic origin of the Protozoa may be complex, with the possibility of algal derivation along different lines of descent at different times in the evolutionary past.

The sequence of cytochrome c (C557) from the flagellate, *Crithidia oncopelti*, has been reported⁹ and I present here the proposed sequence of cytochrome c (C558) from the photosynthetic flagellate, *Euglena gracilis* (Fig. 1). The protein was purified and apoprotein was prepared in the same way as cytochrome C557. Enzymic digestion was performed on

1–1.5 μmol quantities of apoprotein and peptides were separated using conventional techniques¹⁰. The dansyl-Edman procedure¹¹ was used to identify all the residues except the tryptophans and the N-terminal region. Spectrophotometric examination¹² indicated two tryptophan residues per mol and there were two Ehrlich-positive tryptic peptides (Gly₂₃-Arg₃₈ and Asn₅₄-Lys₆₆). The tryptophan residues were positioned on the evidence of sub-digests of these two peptides and carboxypeptidase A experiments.

cysteine. The haem appears to be bound through only one covalent link leaving one vinyl group unsaturated. Cytochromes C557 and C558 are, at present, unique amongst haemoproteins in having this prosthetic structure.

Cytochromes C557 and C558 are not closely related. Out of 103 positions compared, 48 are different. Nor is cytochrome C558 close to wheat cytochrome c; they have 52 differences. Table 2 suggests that a saturation effect occurs at large evolutionary distances and that the cytochrome c molecule becomes

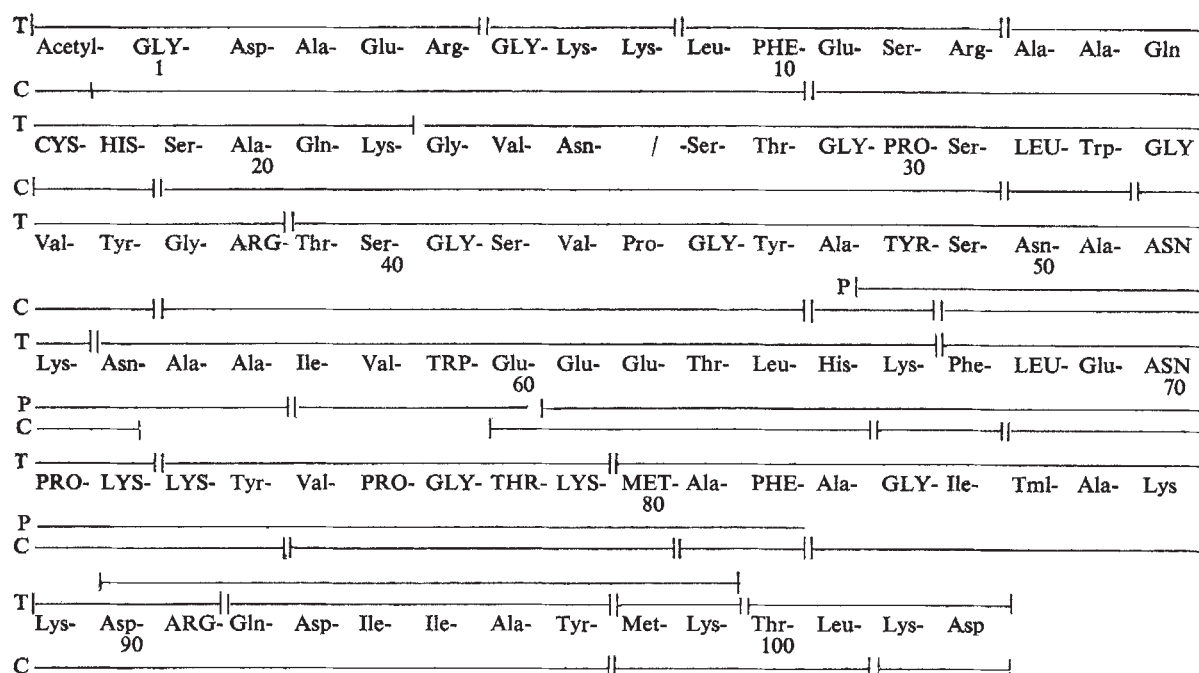


Fig. 1 Proposed structure of cytochrome c from *Euglena gracilis*. Peptides from tryptic (T) and chymotryptic (C) digests are shown. Invariant sites are in capital letters. Tml = Trimethyllysine. / = deletion. Some peptic peptides (P) are included.

The peptide (Gly₂₃-Arg₃₈) did not always give clean results during Edman degradation, due perhaps to acidolysis in the Ser-Thr-Gly sequence. Because of the deletion and the tryptophan in this peptide several independent proofs of its sequence were obtained. The blocked N-terminal peptide was partially hydrolysed in acid and the products were separated and identified. The acetyl group was confirmed after hydrazinolysis as dansyl-acetyl-hydrazine¹³.

Some regions of single residue overlap between tryptic and chymotryptic peptides were confirmed by digestion of the protein with pepsin. The relevant peptides are included in Fig. 1 and are assigned on the basis of amino-acid composition, N-terminal and C-terminal studies only. Amides were located by examining the mobilities of parent peptides, of sub-digest peptides or of remainder peptides during Edman degradation. The amino-acid composition of the protein is in good agreement with the sequence (Table 1).

The mid-point potentials of cytochrome C557 and C558 were measured by the method of Davenport and Hill¹⁴ with ferro-ferricyanide buffers in an anaerobic cuvette. *Pseudomonas* C₅₅₁ and horse heart cytochrome c gave values in fair agreement with literature results (284 mV compared to 282 mV found by Horio¹⁵ for C₅₅₁ and 267 mV compared to 261 mV found by Schejter¹⁶ for the horse heart protein). Cytochrome C557 was determined as 264 mV and C558 as 244 mV.

Like cytochrome C557, the *Euglena* protein has distinctive spectral properties^{6,7}. Meyer has already suggested an atypical haem binding on the basis of the pyridine ferro-haemochrome which has an absorption maximum at 553 nm. This is intermediate between the 550 nm of all other c-type cytochromes and the 556–558 nm of proteins containing protohaem IX.

Residue 14 in cytochrome C558 is alanine in place of

unreliable in defining times of divergence. The structural constraints imposed by functional requirements apparently make this saturation level about half the total number of residues.

The N-terminal sequences and the pattern of methylation of

Table 1 Amino-acid Composition of *Euglena gracilis* Cytochrome c

	Composition	Sequence
Aspartic acid	9.2	9
Threonine	4.85	5
Serine	6.85	7
Glutamic acid	9.0	9
Proline	4.25	4
Glycine	9.65	10
Alanine	11.8	12
Valine	5.0	5
Methionine	1.95	2
Isoleucine	3.95	4
Leucine	4.95	5
Tyrosine	4.8	5
Phenylalanine	3.0	3
Histidine	1.95	2
Lysine	11.9	12
Trimethyllysine	1.0	1
Arginine	4.1	4
Cysteic acid	0.9	1
Tryptophan	1.9	2

Composition based on residues/iron atom and compared with total residues in the proposed sequence.

Aliquots of the same solution were used for 20 and 90 h hydrolyses and for iron determination²¹. Cysteic acid was estimated separately after removal of the haem and performic acid oxidation. Tryptophan was estimated by the Goodwin and Morton method¹².

cytochrome c roughly fit phylogenetic categories. The cytochromes c of vertebrates are acetylated at Gly₁; those of invertebrates and ascomycetes have free and slightly extended N-termini; and those of plants are acetylated at Ala₋₈. Trimethyllysine was found at residues 72 and 86 in plant cytochromes c, 72 in ascomycete cytochromes c and not at all in others¹⁷.

Table 2 Amino-acid Differences

	Horse	Wheat	<i>Neurospora</i>	Yeast	<i>Euglena</i>	<i>Crithidia</i>
Horse	0	46	46	46	45	46
Wheat	46	0	54	47	52	48
<i>Neurospora</i>	46	54	0	41	48	58
Yeast	46	47	41	0	56	51
<i>Euglena</i>	45	52	48	56	0	48
<i>Crithidia</i>	46	48	58	51	48	0

Only those positions common to all were compared.

Neither cytochrome C557 nor C558 fits into these categories. The former has a nine residue extension which is blocked at the amino terminus and residues 8 and 72 are methylated. The latter is acetylated at Gly₁ like the vertebrate cytochromes and Lys₈₆ is methylated.

The deletion at residue 25 in cytochrome C558 is the first example of an internal deletion in a cytochrome c and nearby Lys₂₇ is replaced by a serine. The X-ray structure shows this region as a hairpin loop¹ and perhaps the folding of this loop in cytochrome C558 is different from that of horse heart.

The elucidation of the sequence of cytochrome C557 reduced the number of common residues in the cytochrome c family from 33 to 29. Cytochrome C558 has a novel variation at residue 67 where a phenylalanine replaces a tyrosine. This is the tyrosine that is preferentially nitrated in horse heart cytochrome¹⁸. Three changes which were new variations in cytochrome C557 also appear in C558. Lys₂₇ is replaced by Ser, Ile₇₅ by Val, and Cys₁₄ by Ala.

There are now 28 common sites, four below the minimum proposed by a number of people on statistical grounds or guesswork. Dayhoff¹⁹ predicted 14 which could prove to be a more realistic estimate.

Cytochromes c are claimed to be functionally equivalent on three grounds; their ability to restore respiration in mitochondria depleted of their own cytochrome c²⁰, their reactivity with cytochrome oxidase²⁰, and the thermodynamic parameters of oxidation and reduction¹⁶. This evidence has been used to support an interpretation of cytochrome c evolution by neutral mutations. However, Hill *et al.*³ have shown that *Crithidia fasciculata* cytochrome c reacts more slowly than other eukaryotic cytochromes c with mammalian cytochrome oxidase.

The redox potential of 244 mV for cytochrome C558 determined in this work differs greatly from the 307 mV reported by Perini *et al.*⁶. There is no obvious explanation for this discrepancy. It does appear, however, that cytochrome C558 has a substantially different redox potential from the other mitochondrial cytochromes c studied. Cytochrome C558 thus may not be a member of the functionally equivalent group proposed by Margoliash *et al.* Such an assumption of equivalence may not be justified in any case since too little is known of the detailed role of cytochrome c. The protein cannot be tested thoroughly enough *in vitro* to be confident that changes in its structure are selectively neutral.

These results suggest that cytochromes c may not be so invariant in structure as was anticipated. One of the residues which bind the haem group may change and three positions in the long conserved region preceding Met 80, the sixth haem ligand, are also variable. *Euglena gracilis* cytochrome c may also have slightly different enzymic properties.

One of the possibilities mentioned in the introduction, a homology link with prokaryotes, was not realized. Work is proceeding in this laboratory on cytochrome *f* from *Euglena*

and from *Porphyra tenera* and preliminary results suggest that this protein may provide such a link (R. P. Ambler and G. Pettigrew, unpublished observations).

I thank Dr Richard Ambler and Dr Terry Meyer for encouragement and guidance, Dr A. P. Ryle for commenting on the manuscript, and Steve Hughes and Tam Bruce for growing the *Euglena*. The work was carried out in the laboratories of Professors R. B. Fisher and M. R. Pollock. Professor W. M. Fitch in Wisconsin has been concerned with the same problems and we have usefully exchanged results. I thank Professor Fitch and Dr G. E. Tarr for their cooperation.

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The Properties and Amino-acid Sequence of Cytochrome c from *Euglena gracilis*

CYTOCHROME c, by virtue of its relatively slow evolution and the large number of sequences already determined, is particularly well suited for investigating the phylogenetic relationships among major taxonomic groups, including the relationship of the protozoans to the metazoans. Among the metazoans, more than fifty cytochromes c have been sequenced from fungi, plants, insects and vertebrates. Already one protozoan sequence has been determined, that of *Crithidia oncopelti*¹, and here we describe a second, from the protozoan, *Euglena gracilis*.

A strain of *Euglena gracilis* was grown in the dark at 25° C with rotary shaking in 6 l. Erlenmeyer flasks containing 2 l. of Difco *Euglena* broth supplemented with 1% (w/v) glucose. The cells freed from chloroplasts were harvested at end of log phase growth by centrifugation for 5 min at 1,000g. Generation time was about 9 h. Recoveries were from 30–50 g wet weight l.⁻¹

of medium. Cells were also grown at 30° C in 150 l. batches on a medium containing 1.5% (w/v) glucose, 0.8% (w/v) soy peptone (any peptone seems to work) and salts as defined by Greenblatt and Schiff² (G. E. Tarr, personal communication). The pH was adjusted to 3.8 with concentrated HCl and aeration and mixing accomplished by sparging with air. Yield was 25 g (wet weight) l.⁻¹. Cells were frozen immediately after collection.

Table 1 Composition of *Euglena gracilis* Cytochrome c 558

Amino-acid	20 h hydrolysis	Sequence
Alanine	11.4	12
Arginine	3.5	4
Aspartate	} 9.9	4
Asparagine		5
Cysteine	—	1
Glutamate	} 9.4	6
Glutamine		3
Glycine	11.6	10
Histidine	1.9	2
Isoleucine	2.9	4
Leucine	5.4	5
Lysine	12.6	13
Methionine	1.6	2
Phenylalanine	3.6	3
Proline	4.2	4
Serine	7.2	7
Threonine	5.1	5
Tryptophan	—	2
Tyrosine	3.3	5
Valine	5.5	5
Total	99.1	102

The 20 h hydrolysis set arbitrarily to total 99 residues. Isoleucine is low because of the resistance if Ile-Ile bond between residues 94 and 95.

15 kg of cells was thawed and suspended in 3 volumes of 8 mM, pH 7, NaPO₄ buffer containing 0.2 M NaCl. Cells were disrupted by sonication of 800 ml. aliquots in the presence of 5 µm glass beads. Breakage was > 80% within 15 min. The sonicated suspension was brought to pH 4.2 with glacial acetic acid, let stand for 30 min and centrifuged. The supernatant was adjusted to pH 7 with concentrated ammonium hydroxide, ammonium sulphate added to 55% saturation, let stand for 1 h, centrifuged and the supernatant dialysed and recentrifuged. The cytochrome c of the dialysed supernatant was batch-absorbed on 'Amberlite CG-50', washed with pH 7, 0.02 M NaPO₄ buffer, then eluted with the same buffer but containing 0.5 M NaCl and the volume reduced by pressure dialysis. The procedure beginning with ammonium sulphate was repeated except that it was brought to 60% saturation, the cytochrome oxidized with a few grains of K₃Fe(CN)₆ before applying to the 'Amberlite', and a linear ionic gradient used for elution. The cytochrome appeared at a salt concentration of 0.28 M although this will vary, not only as a function of the pH, but also as a function of the ratio of column volume to buffer volume in the mixing chambers. Gel filtration on 'Sephadex G-75' gave minimal improvement. The cytochrome, in 5 mM Tris-HCl buffer pH 8.37, was applied to a DEAE-cellulose column and eluted with the same buffer. The peak fraction had an OD₅₅₈/OD₂₈₀ of 0.85 and had properties similar to the preparation of Perini *et al.*³. Isoelectric focusing was performed with a pH 8–10 ampholyte at a final concentration of 0.5% (w/v) plus arginine at 0.1% (w/v) using three different sucrose gradients with a 40% range. The cathode was placed on the bottom with 1% (v/v) monoethanolamine, 1% (w/v) Tris and 1% (w/v) N-ethylmorpholine in 75% (w/v) sucrose while the anode was in phosphoric acid (1/100 v/v) 1 in 100. Cytochrome c was applied in the reduced (by dithionite) form. The column was equilibrated 48 h at a maximum of 1,500 V. Only one band of cytochrome c was obtained and it represented no improvement in purification over the DEAE-cellulose treated product. The isoelectric point was determined by linear extrapolations to zero sucrose concentration. All of the above procedures were carried out at 4° C except the iso-electric focusing which was at 0° C.

A summary of the amino-acid composition and physical-chemical characteristics of cytochrome c is shown in Tables 1 and 2. Ultracentrifugation was performed at 2 concentrations differing by a factor of 10. We have recently also purified *Euglena* cytochrome c by the method of Margoliash and Walasek⁴.

The derived amino-acid sequence is shown in Fig. 1. The number of residues that are absolutely necessary for cytochrome c function is of interest. An attempt to estimate this number, using the distribution of substitutions in codons required in their descent to account for known present day sequences, was presented by Fitch and Margoliash⁹. Their results suggested that about 30 of the 35 unvaried positions were invariable. Fitch and Markowitz¹⁰ showed, however, that the number of invariable positions depended on the range of species examined. Within that range, further sequences have since revealed only two additional residues to have had substitutions that survived the evolutionary selective processes, Ala₅₁→Gly in the ginkgo tree¹¹ and Tyr₇₄→Phe in a thermophilic fungus *Humicola*¹². The present sequence, along with that of *Crithidia*, is, however, outside that range, and according to the concept of Fitch and Markowitz might be expected to show variability in previously unvaried residues. This proves true as follows: *Euglena* Tyr₆₇→Phe, Ile₇₅→Val, and Lys₈₇→Ala (also *Physarum* (Lin, Niece and Fitch, unpublished)) *Euglena* and *Crithidia* Cys₁₄→Ala, Lys₂₇→Ser, Gly respectively; also the trivially unvaried position, 9 (only known previously in *Saccharomyces*, Ala) is Pro in *Crithidia*. These six, plus the two above, reduce the number of unvaried positions to 27. For this wider range of species the estimate of the invariable codons is reduced to 24 ± 5, confirming the effect of species range.

Table 2 Properties of *Euglena gracilis* Cytochrome c 558

Molecular weight	
Gel filtration ('Sephadex G-75')	12,600 ± 800
Sedimentation equilibrium ('Spinco' model E, 60,000 r.p.m. 20°)	10,940
Sequence analysis	11,254
Spectral characteristics	
Maxima (oxidized)	278, 365, 411, 530 nm
Maxima (reduced)	275, 320, 422, 525, 558 nm
mMolar extinction coefficient at 558 nm	25.7
Iso-electric point	9.55

The supposed invariability of several of the altered residues had been attributed to their presumed essential function. Cys₁₄ was considered necessary for the attachment of the haem. Its absence leaves an additional vinyl group conjugated with the haem system of double bonds and must be the major reason for the shift in the reduced maximum in the protozoan cytochrome to 558 nm. The two residues, Tyr₇₄ (*Humicola* Phe) and Ile₇₅ (here Val), are both part of a stretch of eleven consecutive residues not previously known to vary and considered part of a recognition site for other proteins¹⁴. Tyr₆₇ has been suggested to be involved in the passage of electrons in and out of the haem^{15,16}. Much previous speculation must therefore be reconsidered in light of these findings.

Regarding the relationship between *Euglena*, *Crithidia*, and the metazoans, we conclude that the two protozoans are more closely related to each other than either are to the metazoans. This conclusion requires the examination of the messenger sequences of the proteins and the elimination of all non-critical positions. A non-critical position is a position for which the minimum number of nucleotide replacements required is independent of the phylogeny. For example, at codon 20, *Euglena* has alanine, *Crithidia* glycine, *Neurospora* glutamate, and pig, cotton and *Drosophila* all have valine. The central nucleotides of these codons must be C, G, A, U, U and U respectively. Because only one nucleotide appears more than once, then the minimal number of mutations is one less than the number of kinds of nucleotides regardless of the phylogeny assumed (4 – 1 = 3 in this position) and all mutations required by the data will be peculiar to the final leg descending to the exceptional taxon. Having eliminated the non-critical positions, we may then ask: what is the minimum pairwise distance (disjointness) between the species in the remaining critical

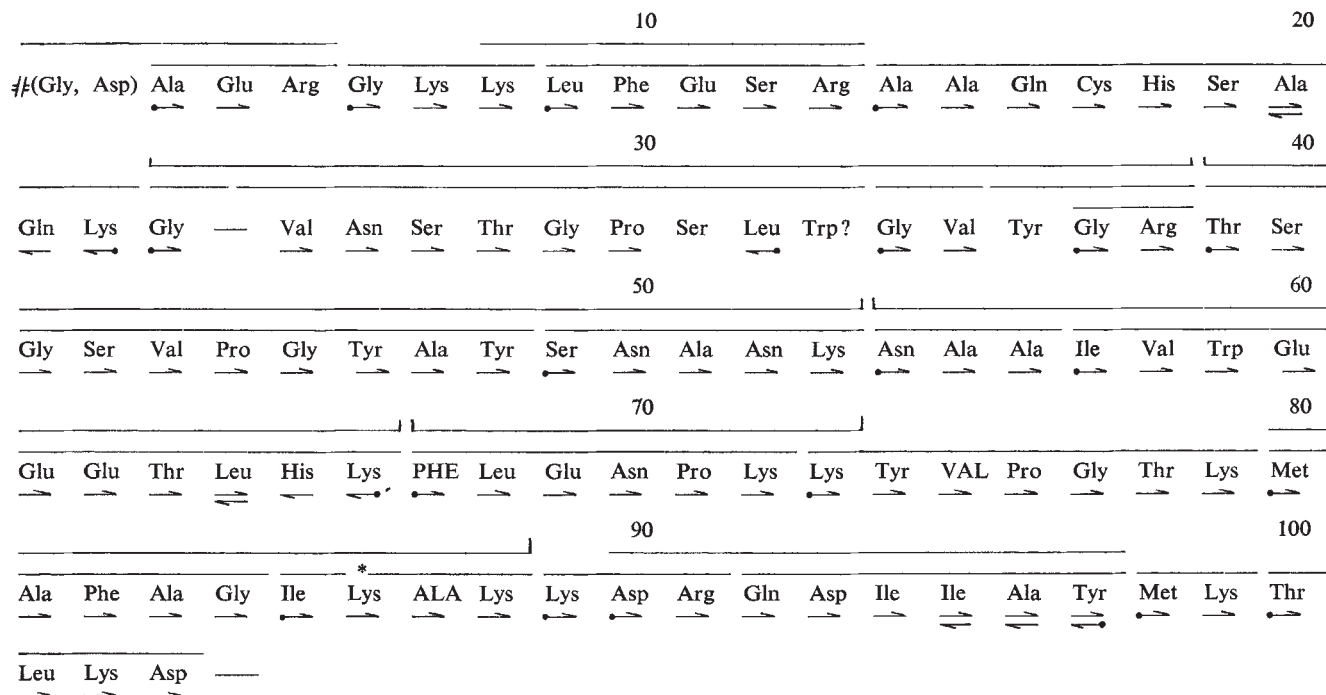


Fig. 1 Amino-acid sequence of *Euglena gracilis* cytochrome c. The solid lines above indicate peptides isolated and sequenced. The solid lines with tick marks on their ends indicate peptides whose composition spans sequenced peptides. The $\rightarrow$ and $\leftarrow$ arrows indicate residues determined by PTH-Edman and carboxypeptidase A or B procedures. The dot on the end of an arrow indicates that it is the first residue found by that procedure. The gaps at positions 24 and 104 are simply to show the alignment relative to other eukaryotic cytochromes c. The amino terminus (#) is blocked, presumably by acetylation. Lys 86 (*) is trimethyl lysine. Position 33, (Trp?), was never identified directly, only spectrally and by Ehrlich reaction in this peptide. Positions previously unvaried (67, 75 and 87) are shown in capital letters. Tryptic digestion was carried out in 0.1 M NH_4HCO_3 for 5.5 h at 40° C with the cytochrome concentration at 1 % and TCPK-trypsin at 0.02 %. The tryptic peptides were separated by pH gradient elution on 'Dowex AG-50W X₂' according to the method of Schroeder⁵. The tryptic peptides were repurified as necessary by paper chromatography. The amino-acid sequences of the peptides were determined by the method of Edman and Begg⁶ as modified by Keresztes-Nagy, Perini and Margoliash⁷. PTH-derivatives were identified as fluorescence quenching spots after silica-gel chromatography in solvents IV and V of Jeppson and Sjöquist⁸.

positions, that is, the only positions that, depending upon the phylogeny, could require parallel and back mutations? The results are shown in Table 3. Obviously, by eliminating substitutions peculiar to a single line, one tends to eliminate them most frequently from lines whose most recent ancestor common to the other taxa was longest ago. Thus "distance" is misleading and is the reason for using the term disjointness. It is clear that the two protozoans are less disjoint with respect to each other than either of them are to the metazoans. In this case, the number of possible unrooted phylogenies is only 105 and all 105 of them were tested and it was found that the fewest mutations were required in a phylogeny joining *Euglena* and *Crithidia*. A proper assessment of their relationship will better be made when additional protozoan sequences become available.

The work reported here was done independently of that of Pettigrew¹³ with whom we agree as to the sequence of *Euglena gracilis* cytochrome c 558. It is our great pleasure to note the totally open communication with him since we became aware

of our parallel pursuits. The assistance of Mr Frank Iltis and Dr George Tarr is recognized. This work was supported by a grant to W. M. F. from the US National Science Foundation.

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Table 3 Minimum Pairwise Disjointness

	1	2	3	4	5	6
1 Pig	—					
2 <i>Drosophila</i>	17	—				
3 Cotton	39	43	—			
4 <i>Neurospora</i>	35	30	34	—		
5 <i>Euglena</i>	38	42	41	33	—	
6 <i>Crithidia</i>	43	38	33	34	26	—

The minimum pairwise disjointness is the number of nucleotides by which the mRNAs for cytochrome c from two species must differ in those nucleotide positions for which there are two or more different nucleotides appearing two or more times each. See text for explanation.

Developmental Maturation of Susceptibility to Scrapie in Mice

AGE at the time of injection has not been shown to influence the incubation period of peripherally injected scrapie in mice over 6 days old¹, although a slight negative regression from birth to four months has been found for the intracerebral route². We report here, marked incubation period changes of three scrapie agents given intraperitoneally when the mice were injected neonatally instead of as adults.

Table 1 The Incubation Periods Shown by Weanling Mice after an Intraperitoneal Injection of Various Strains of Scrapie Agent

Agent (mouse-passaged)	Inbred mouse strain	Dilution of infected brain	Incubation periods $\pm$ s.e. (days) in weanlings*	
			Females	Males
22A	VM	10^{-2}	319 ± 6	355 ± 5
79A	VL	10^{-2}	205 ± 1	215 ± 2
ME7	C57BL	10^{-1}	225 ± 4	233 ± 3
		10^{-2}	249 ± 2	250 ± 4
		10^{-3}	278 ± 6	275 ± 3

* Weaned at 21 days old.

The inocula were 10^{-1} , 10^{-2} or 10^{-3} supernates (500 g, 10 min) of saline mouse-brain homogenates from terminal cases with ME7, 79A and 22A strains of scrapie³⁻⁶. Each agent was injected (0.02 ml.) intraperitoneally into C57BL, VL or VM mice, ranging in age from birth to 28 days. Any leakage of inoculum in 0 to 6 day old mice was minimized by inserting the fine needle (32G) anterior to the xiphisternum, through a long subcutaneous track, and penetrating the peritoneum adjacent to the liver, which assisted as a non-return valve. The injection site was sealed immediately with 'Nobectane' acrylic solution. Table 1 gives incubation periods⁴ for weanling mice with these agent/host strain combinations. Animals showing no clinical signs or histological lesions of scrapie, and which died or had to be killed over 550 days of age, were regarded as survivors. Depending on the particular agent/host combination, these survivors exceeded the mean incubation period for weanlings by more than 10 to 50 standard deviations.

Injection of very young mice frequently produced some survivors and a proportion of much longer or appreciably shorter incubation periods than those observed in weanling mice (Table 2). Cases following neonatal injection with ME7 agent tended to occur earlier with high titre inocula but later with low titre ones than in the weanling group, and survivors only occurred in the newborn group given the lowest titre. The proportion of survivors using 10^{-2} homogenates was higher with the 22A and 79A agents than with ME7. It must be emphasized that survivors do not occur in weanlings using these doses, either in these or in large numbers of other comparable experiments.

Several explanations for this phenomenon are relevant to the early pathogenesis of scrapie following peripheral inoculation. Neonatal mice probably lack some cell populations required for the initiation of pathogenesis. Hypotheses about the cause of the age-effect need to take account of the paradox that these data would give lower titre estimates using neonatal mice than using weanlings but with shorter incubation periods than weanlings at high doses. Two competing conditions may be present in the host, one favouring replication, the other destruction, of agent, and the outcome would depend on the balance of an individual's developmental state and the dose of agent.

The earliest replication of scrapie agent is known to take place in lymphoreticular tissue⁷, indicating this to be the relevant system which is maturing in mice during the first postnatal week. There are numerous cell types in this tissue whose immaturity is involved in neonatal immunological immaturity^{8,9} and age-dependent susceptibility to virus infections^{10,11}. These age-dependent effects with scrapie are quite different from those with conventional viruses and, if due to lymphoreticular maturation, are unlikely to implicate either specific immune reactions or interferon production as these appear to play no part in the pathogenesis of scrapie¹²⁻¹⁶.

The occurrence of survivors in mice given doses which would be lethal for all weanlings, raises the question of what happens to the agent in these individuals. There is no realistic possibility that leakage of inoculum can explain these results with the precautions we used, because even prolonged incubation periods would require the leakage of nearly all of it. The remarkable loss in effective titre, in conjunction with the

Table 2 The Distribution of Incubation Periods* of Three Scrapie Agents Injected i.p. in Mice of Different Ages

Agent (mouse-passaged)	Inbred mouse strain	Dilution of infected brain	Age (days)	Number of mice	Numbers of individuals with incubation periods in SD groups less than (-) or more than (+) the mean for weanlings.						Survivors†
					> 3 SD	-3 to -2 SD	-2 to +2 SD	+2 to +3 SD	> 3 SD		
22A	VM	10^{-2}	21-28	27	0	2	24	1	0	0	0
			4-6	16	2	0	9	1	1	3	3
			1	8	0	0	0	0	0	8	8
			0	10	1	0	0	0	0	9	9
79A	VL	10^{-2}	20-28	37	0	1	34	2	0	0	0
			6-7	28	0	6	15	5	2	0	0
			2-3	15	0	0	5	6	4	0	0
			0-1	24	1	1	0	2	11	9	9
ME7	C57BL	10^{-1}	21-28	8	0	1	7	0	0	0	0
			7-8	6	2	0	4	0	0	0	0
			0	17	7	3	2	0	5	0	0
		10^{-2}	21-28	20	0	0	19	1	0	0	0
			4-8	31	3	4	10	5	9	0	0
			2	7	1	3	2	1	0	0	0
		10^{-3}	0-1	31	0	4	18	1	8	0	0
			21-28	11	0	0	10	1	0	0	0
			8	8	0	0	2	1	5	0	0
			3	5	0	0	0	0	5	0	0
			0	6	1	0	0	1	2	2	2

* The individual incubation periods are classified in terms of standard deviations (SD) from the mean incubation period in weanling mice.

† Mice more than 550 days old, died or killed, with no signs of scrapie.

tentative conclusion that newborn mice lack one or more relevant sets of receptor cells, raises three possibilities for the fate of the agent. It could be removed either by excretion (for example, the neonatal gut is permeable at least to proteins), or by sequestration in tissues where it cannot replicate, do damage or gain access to target cells, or it could be inactivated enzymically, possibly by macrophages. Cases with very short incubation, in contrast, could result from high doses of agent overloading the hypothetical disposal systems, thus leaving some agent available when the receptors mature. This could be earlier in some individuals than others.

These experiments may explain maternal transmissions in sheep¹⁷ and its absence in mice¹⁸, because sheep are born with relatively more mature lymphoreticular responses¹⁹. This implies that the postulated receptors, though absent in newborn mice, are present in the sheep foetus.

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In vitro Morphine Antagonism by Antibodies

MORPHINE has a variety of pharmacological effects including inhibition of motility of some types of smooth muscle^{1,2}. The narcotic antagonists nalorphine and naloxone appear to reverse a majority of the pharmacological effects of morphine by competitive inhibition for the tissue receptor site^{1,2}. Our study was designed to determine if antibodies reactive with morphine could also inhibit or successfully reverse one of the biologic actions of the drug. We examined the capacity of rabbit antibodies produced by immunization with 6-succinyl-morphine conjugated to bovine serum albumin (M-6-HS-BSA)^{3,4} to antagonize the depressant action of morphine on electrically-induced contractions of isolated guinea pig ileum.

A concentrated crude globulin fraction of hyperimmune serum obtained from a rabbit immunized with M-6-HS-BSA

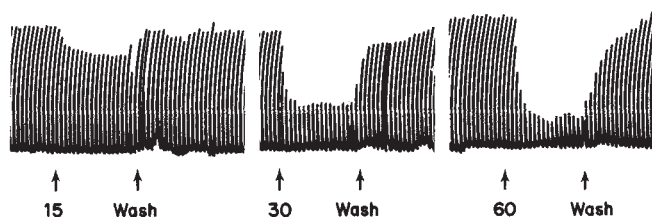


Fig. 1 Effect of 15 nM, 30 nM, and 60 nM morphine concentrations on the electrically-induced contractions of isolated guinea pig ileum. The amount of morphine added is expressed in terms of the final molar drug concentration in the bath that is 60 nM represents 0.6 nmol added to the 10 ml. bath. The drug was added to the muscle bath for 3 min periods and then washed out. There was an interval of at least 15 min between the addition of different drug concentrations.

was prepared by precipitation with 50% ammonium sulfate followed by pressure dialysis. The reactivity of the antiserum has been studied in detail⁵. Concentrations of various opioids inhibiting binding of 88 pmol ml⁻¹ of ¹⁴C-morphine by 50% were; heroin, 0.10 nmol ml⁻¹; morphine, 0.11 nmol ml⁻¹; codeine, 0.16 nmol ml⁻¹; hydromorphone, 0.50 nmol ml⁻¹; nalorphine, 2.1 nmol ml⁻¹; meperidine, 80 nmol ml⁻¹; and naloxone, 30% inhibition at 1 × 10³ nmol ml⁻¹. The concentrated globulin fraction bound 91,000 pmol morphine ml⁻¹ as determined by the ammonium sulfate method^{6,7} when the concentration of added antigen was 88 pmol ml⁻¹ ¹⁴C-morphine. Crude globulin from normal rabbit serum (NRS) and from rabbit antiserum reactive with BSA (anti-BSA) were prepared and concentrated by the same procedures. Specific binding of ¹⁴C-morphine was not detected in either serum before or after ammonium sulfate fractionation. The protein concentration of each crude globulin fraction was adjusted to 30 mg ml⁻¹.

The assay for morphine activity was based on the depressant action of morphine on the electrically stimulated contractions of the longitudinal muscle of guinea pig ileum^{8,9}. The ileal segment was stimulated coaxially with 1.5 times maximal rectangular pulses of 0.8 msec duration at a frequency of 6 pulses min⁻¹, and the muscle twitch was measured isometrically with an "E&M Model Six physiograph" and "Type A pressure transducer". There was an interval of at least 15 min between additions of different drug mixtures to the bath.

The inhibitory effect of increasing concentrations of morphine hydrochloride on the muscle twitch is shown in Fig. 1. The drug was allowed to remain in the bath for 3 min and then washed out. Inhibition was observed immediately upon addition of morphine to the bath and this inhibition was reversed on removal of the drug by washing. The lowest concentration at which this effect could be detected was at 15 nM morphine. Inhibition was 70–80% complete at 60 nM morphine concentration.

The capacity of anti-M-6-HS-BSA to antagonize the depressant actions of morphine on the ileal segments was measured. Varying concentrations of morphine were preincubated with 0.4 ml. of antibody globulin for 15 min at room temperature, and then added to the tissue bath for 3 min. This amount of antibody inhibited the effect of morphine concentrations ranging from 60 nM to 1,000 nM (Fig. 2). Morphine preincubated with NRS globulin retained full activity, and addition of antibody or NRS globulin alone had no effect on the contractility of the ileal muscle. This prevention of the pharmacological effect of morphine on isolated guinea pig ileum was probably due to the morphine-antibody combination stopping the morphine reaching the biological receptor sites. Prevention of the morphine effect was complete when a two-fold stoichiometric excess of antibodies was present.

To determine if antibody was able to reverse morphine

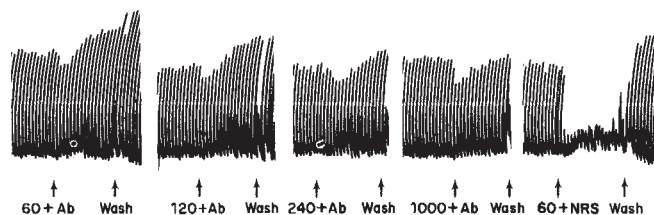


Fig. 2 Effect on the electrically-induced contractions of isolated guinea pig ileum of adding 0.4 ml. anti-M-6-HS-BSA globulin (Ab) preincubated with either 60 nM, 120 nM, or 1,000 nM morphine; and the effect of adding 0.4 ml. normal rabbit globulin (NRS) preincubated with 60 nM morphine.

action, antibody was added to the muscle bath 1 min after addition of morphine when the induced inhibition of muscle contraction was maximal. Typical results are illustrated in Fig. 3a. Anti-M-6-HS-BSA added for 1 min after morphine completely reversed the inhibition of muscle twitch caused by 120 nM morphine. The recovery of the twitch response was complete within 2 min and this effect appeared to be identical to that observed after addition of 10 nM naloxone. (Fig. 3b). Neither NRS globulin or anti-BSA globulin (Fig. 3c) had any effect on the morphine-induced inhibition of the muscle twitch. Reversal of the morphine effect was about 80% complete when a stoichiometrically equivalent amount of antibody was added to the muscle bath.

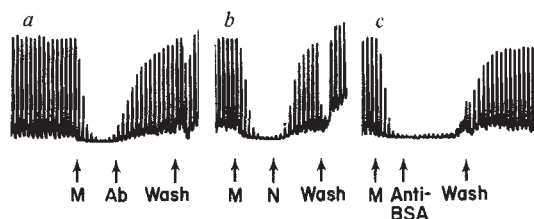


Fig. 3 Effect on the electrically-induced contractions of isolated guinea pig ileum of 120 nM morphine (M) present in the muscle bath for 1 min followed by the addition of either: a, 0.5 ml. of anti-M-6-HS-BSA (Ab); b, 10 nM naloxone (N); or c, 0.5 ml. of anti-BSA.

Similar experiments have been carried out with globulin obtained from several rabbits immunized with morphine-6-succinyl-BSA, with identical results. We suggest that because anti-BSA globulin has no effect on the morphine activity the inhibition of biological action of morphine is the specific effect of antibodies reactive with morphine.

Results of our experiments indicate that sepecific antibodies are capable of antagonizing the inhibitory effect of morphine on the electrically stimulated contractions of guinea pig ileum. The antibody molecules apparently bind morphine more strongly than the tissue receptor site as antibodies were able to prevent and reverse the inhibitory effect of morphine on smooth muscle contraction. The use of this sensitive *in vitro* assay together with immunochemical measures of antibody avidity should allow determination of the molar concentrations of antibody required for morphine antagonism and the relative affinities of morphine receptors in tissues for their drug substrates.

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Glucose Catabolism by the Simian Malaria Parasite *Plasmodium knowlesi*

ALTHOUGH the parasitology of malaria has been extensively studied¹, biochemical aspects of the relationships between the intra-erythrocytic parasite and its host erythrocyte are poorly understood. The main source of metabolic energy for the erythrocytic stages of the parasite is the breakdown of glucose with formation of lactic acid²⁻⁶, which it may carry out independently of the red cell². Catabolism of glucose by metabolic pathways other than glycolysis may occur, although the overall pattern of glucose breakdown appears to vary with the species of malaria parasite⁷. The ability of the parasite, as distinct from the erythrocyte, to carry out the reactions of the pentose phosphate pathway is unresolved^{8-11,19}.

Here we have attempted to estimate the flux of glucose through the glycolytic pathway, in normal rhesus monkey erythrocytes, in erythrocytes infected with *P. knowlesi* and in parasites "freed" from their host erythrocytes. *P. knowlesi* shows synchronous growth with a 24 h asexual life cycle and parasites may therefore be conveniently prepared from the monkey host in relatively large quantities at well defined life

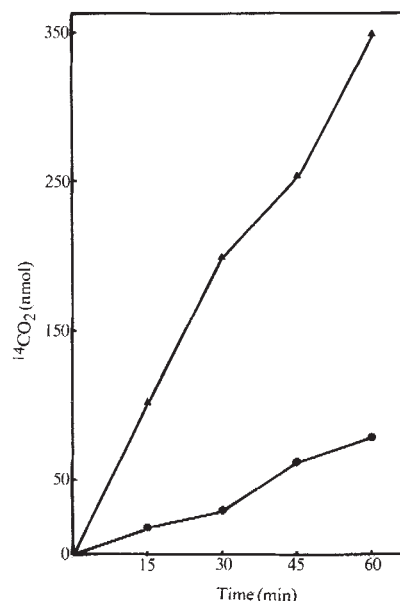


Fig. 1 Production of ¹⁴CO₂ from 1-¹⁴C- and 6-¹⁴C-D-glucose in erythrocytes of the rhesus monkey infected with *Plasmodium knowlesi*. ▲, 1-¹⁴C-D-glucose; ●, 6-¹⁴C-D-glucose.

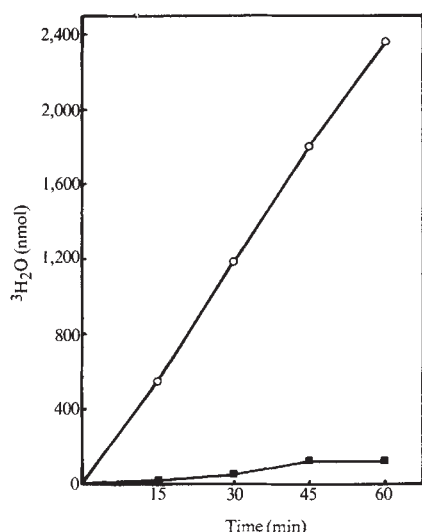


Fig. 2 Release of $^3\text{H}_2\text{O}$ from 2- ^3H -D-glucose by erythrocytes from the rhesus monkey. ■, Normal; ○, infected with *Plasmodium knowlesi*.

cycle stages. Parasites were prepared when they had grown to the late trophozoite and schizont stages.

The flux of glucose through the glycolytic pathway and the primary dehydrogenases of the pentose phosphate pathway was measured radiochemically by the method of Hutton¹² which estimates glycolytic flux as the release of $^3\text{H}_2\text{O}$ from 2- ^3H -D-glucose in the hexosephosphate isomerase (EC 5.3.1.9) reaction. Flux through the primary dehydrogenases of the pentose phosphate pathway was estimated from the release of $^{14}\text{CO}_2$ from 1- ^{14}C -D-glucose and 6- ^{14}C -D-glucose.

The results (Figs. 1 and 2) show that the release of $^3\text{H}_2\text{O}$ from 2- ^3H -D-glucose and of $^{14}\text{CO}_2$ from 1- ^{14}C - and 6- ^{14}C -D-glucose were linear over the experimental time. The flux of the glucose through the glycolytic pathway obtained by radiochemical investigation agreed with the values obtained by measuring production of lactic acid, and was greatly increased in the parasitized erythrocytes as compared with normal erythrocytes (Table 1). A high value for glycolytic flux was observed in the free parasites.

The results presented in Table 1 show also that the flux of glucose through the primary dehydrogenases of the pentose phosphate pathway was very much greater in parasitized

erythrocytes than in normal erythrocytes. This large increase in metabolic activity was retained with the parasite when "freed" from its host erythrocyte.

The high $^{14}\text{CO}_2$ production from 6- ^{14}C -D-glucose indicated the presence of an oxidative metabolic process in the parasitized erythrocyte and in the "free" parasite preparation. This value is higher than can be accounted for by recycling through the hexose phosphate pool^{16,17}. Randomization of the C_6 position into the C_1 position of the hexose phosphate pool indicates that the specific activity of the C_1 position may be up to 20% of the initial specific activity of the C_6 position¹⁸. A reasonable estimate of the maximal rate of $^{14}\text{CO}_2$ production from 6- ^{14}C -D-glucose recycling through the pentose phosphate pathway would be about 20% of the rate of $^{14}\text{CO}_2$ production from 1- ^{14}C -D-glucose assuming 100% recycling. Observed values would probably be lower than 20%. Although extensive recycling of hexose phosphate in the erythrocyte has been reported²⁰ it is most unlikely that 100% of the hexose phosphate produced in the pentose phosphate pathway would be recycled by glucose-6-phosphate. Our results show that the rate of production of $^{14}\text{CO}_2$ in the parasitized erythrocyte was between 19–28% of the rate of production of $^{14}\text{CO}_2$ from 1- ^{14}C -D-glucose. In the "free" parasite preparation the corresponding figure lay between 22% and 50%. This suggested that $^{14}\text{CO}_2$ was produced from 6- ^{14}C -D-glucose by processes other than recycling through the pentose phosphate pathway.

We believe that our results represent a valid assessment of the flux through the glycolytic pathway and through the primary dehydrogenases of the pentose phosphate pathway, though the method yields no information of the fate of end products and intermediates of these pathways. Glycolytic flux is measured as the flux through the first enzyme peculiar to the glycolytic pathway. It is an accurate measurement of the flux of glucose through to pyruvate only if the pentose phosphate pathway is of small activity compared to the glycolytic activity. In our system, 20% or less, of the glucose appears to be metabolized by the initial enzymes of the pentose phosphate pathway. We consider that this, together with the agreement between radiochemical measurements of the glycolytic flux and measurement of lactate production provides good evidence that the radiochemical measurement is giving an acceptable assessment of glycolysis and that the method is a valuable tool for the investigation of metabolic characteristics in malaria infection.

Our results show therefore that the breakdown of glucose in the parasitized erythrocyte, by both glycolysis and the pentose

Table 1 Glycolytic Flux and Lactate Production

	$^3\text{H}_2\text{O}$ release nmol/ 10^9 cells h^{-1}	Lactate production nmol/ 10^9 cells h^{-1}	$^{14}\text{CO}_2$ production from: 1- ^{14}C -D-glucose nmol/ 10^9 cells h^{-1}	6- ^{14}C -D-glucose nmol/ 10^9 cells h^{-1}
Normal erythrocytes	75–155 (4)	180–224 (4)	9–16 (3)	Not detected
Parasitized erythrocytes	800–2,360 (3)	1,988–4,912 (5)	348–410 (3)	67–116 (3)
"Free" parasites	1,080–3,600 (3)	None available	368–478 (3)	78–196 (3)

Values given are upper and lower values in the observed range. Number of experiments is shown in parentheses. Both parasitized erythrocytes and free parasites for the radiochemical experiment were prepared from blood from the same infected rhesus monkey. Erythrocytes were prepared from freshly drawn heparinized blood by centrifugation at 1500g for 10 min, washed twice under the same conditions in Krebs glucose saline, and separated from white cells and platelets using a sucrose gradient¹³. Parasites were freed from host erythrocytes by immune lysis¹⁴. Erythrocytes and parasites were washed three times by centrifugation at 1500g for 10 min with the medium used in the glycolytic incubation¹⁵. Erythrocytes and parasites were finally suspended in the glycolytic medium to 50% haematocrit. The cells were incubated in closed Warburg flasks containing: 2.0 ml. glycolytic medium, 0.5 ml. erythrocyte or parasite suspension with 0.5 μCi 1- ^{14}C or 6- ^{14}C -D-glucose and 1.5 μCi 2- ^3H -D-glucose. The reaction was terminated by tipping 0.5 ml. of 20% TCA from the side arm and carbon dioxide evolved was trapped in 0.3 ml. hyamine (Koch-Light Labs, Bucks) in the centre well. The flasks were pre-incubated 15 min at 37°C before addition of the radioactive substrate in 10 μmol of carrier glucose. At the end of the experiment the flasks were further shaken in the 'New Brunswick Controlled Environment Incubator' for 1 h at 50 to 60 cycles per min to remove final traces of $^{14}\text{CO}_2$. Recovery of $^{14}\text{CO}_2$ was calculated by trapping $^{14}\text{CO}_2$ evolved from a known addition of $\text{Na}_2^{14}\text{CO}_3$ after the addition of TCA. This was estimated as $70 \pm 2\%$. $^3\text{H}_2\text{O}$ was sublimed from the incubation mixture under vacuum in Thunberg tubes, trapping the sublimed $^3\text{H}_2\text{O}$ by immersing one end of the tube in a dry-ice-methanol bath. Counting of the $^{14}\text{CO}_2$ was performed in toluene based scintillator (6 g PPO, 0.12 g POPOP l.⁻¹) and the $^3\text{H}_2\text{O}$ was counted in a Dioxan based scintillator (180 g l.⁻¹ naphthalene 4 g PPO, 0.1 g POPOP l.⁻¹). All counting was performed on a 'Packard liquid scintillator spectrometer'. Erythrocyte and parasites were counted using a 'Thoma' haemocytometer with Neubauer ruling after dispersing parasites by drawing through a syringe needle several times.

phosphate pathway, was very much increased compared with the normal erythrocyte. These activities were recovered, at least in part, with the parasite when it was "freed" from its host erythrocyte. We have not made a quantitative assessment of this recovery, nor does this observation indicate that the activity observed is of parasite origin. The immune lysis method used to "free" the parasites may not remove the erythrocyte membrane from the parasite-erythrocyte complex although the host cell haemoglobin was removed, and it is possible that host erythrocyte glycolytic enzymes may be retained in the "free" parasite preparation associated with this²¹⁻²³. It is essential to discover from the point of view of the host-parasite relationship, if the increase in metabolic activity is due to the elaboration of an independent metabolic capability by the parasite, or if the parasite can enhance and utilize the metabolic processes of its host erythrocyte.

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A Preliminary Investigation of the Swine Vesicular Disease Epidemic in Britain

ON December 11, 1972, an outbreak of vesicular disease in pigs was reported from Staffordshire. It was diagnosed as foot-and-mouth disease (FMD) on clinical grounds but this was not confirmed on initial serological tests. Investigations were carried out to determine the nature of the aetiological agent.

Outbreaks of disease have occurred in pigs on two previous occasions which have been clinically indistinguishable from FMD, but from which different causal agents have been isolated. Nardelli *et al.*¹ described such a syndrome in Italy in 1966, and suggested that the agent responsible was a porcine enterovirus, and this has been confirmed by Newman *et al.*². Another outbreak in pigs in Hong Kong in 1971 was reported by Mowat *et al.*³ and the causative agent was again described as a porcine enterovirus (Hong Kong 1971), serologically similar to the Italian enterovirus.

The disease as observed in Britain closely resembles that described in these two reports (A. J. G. Stubbins, R. S. Hedger and G. N. Mowat, personal communication). It occurred only in pigs although on several premises cattle or sheep were in close contact with large numbers of the infected animals. Fifteen outbreaks were confirmed in four weeks, eight being in the first week. Several outbreaks occurred in pigs which were fed on swill collected from the Birmingham area, and in a number of instances spread from the first reported outbreak could be attributed to the movement of pigs or of people. The pattern of disease spread suggested an incubation period of four to six days. Morbidity rates of up to 60% were recorded and the lesions could not be differentiated from those of FMD.

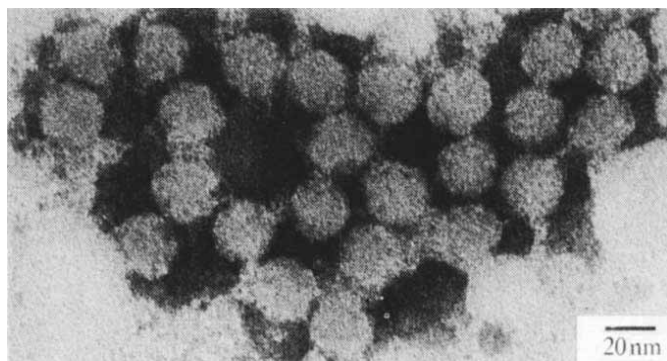


Fig. 1 Electron micrograph of complexes of the swine vesicular disease virus (England 1972) with antiserum to the Hong Kong 1971 enterovirus.

Vesicular fluid, extracts of vesicular epithelium and fluid from infected tissue culture monolayers were tested by complement fixation for the presence of FMD virus or enterovirus antigens. Hyperimmune guinea-pig sera were used which had been prepared against all seven types of FMD virus and against the Italy 1966 enterovirus. The tests were performed in microplates using a fixed complement dose of five 50% haemolytic units and a constant amount of antigen. The antisera were diluted in 1.5-fold series from starting dilutions of 1/16; plates were incubated for 18 h at 4° C. In all of the samples tested there was no reaction with any of the FMD virus antisera. Positive results, however, were obtained with the Italy 1966 enterovirus antiserum, in many instances using material from infected pigs and in all tests in which tissue culture fluid was used from monolayers showing extensive cytopathogenic effects.

To further verify the identity of the virus, virus from infected tissue culture fluid was purified, mixed with serum from a pig convalescent from Hong Kong 1971 enterovirus and examined in the electron microscope ('Siemens Elmiskop I'). Complexes of virus and antibody were seen (Fig. 1), which were also present in mixtures of virus and the Italy 1966 enterovirus antiserum, but were not observed when the virus was mixed with FMD antiserum.

FMD virus differs from enteroviruses in being acid-labile, exposure to pH 5 causing a reduction in infectivity by degradation of the virion to its RNA and protein subunits⁴. Swine vesicular disease virus (England 1972) when so treated showed

no loss of infectivity (J. N. Wilson, personal communication), and electron microscopy showed that after acid treatment, no virions were observed in FMD virus samples but in samples of swine vesicular disease virus the virions remained intact (Fig. 2).

Cell monolayers of primary calf thyroid and BHK 21 tissue cultures inoculated with extracts of vesicular epithelium showed minimal cytopathogenic changes and although virus was isolated from them after 48 h, direct evidence of replication has not yet been obtained. Twenty-four h slope tube cultures of the pig kidney cell line IB-RS-2, inoculated with the same material, showed plaques of round, refractile cells 24 h after infection, which progressed to a total destruction of the cell sheet after 48 h. Virus adapted by serial passage to IB-RS-2 cells grew to titres of up to 2×10^9 p.f.u. per ml. of tissue culture fluid. Primary pig kidney cell monolayers were less susceptible than IB-RS-2, but there was cytopathic effect and virus multiplication.

The presence of the virus in tissue cultures showing typical cytopathic effect was confirmed by complement fixation tests. This cytopathic effect was inhibited by antisera to the Italy 1966 or Hong Kong 1971 enteroviruses.

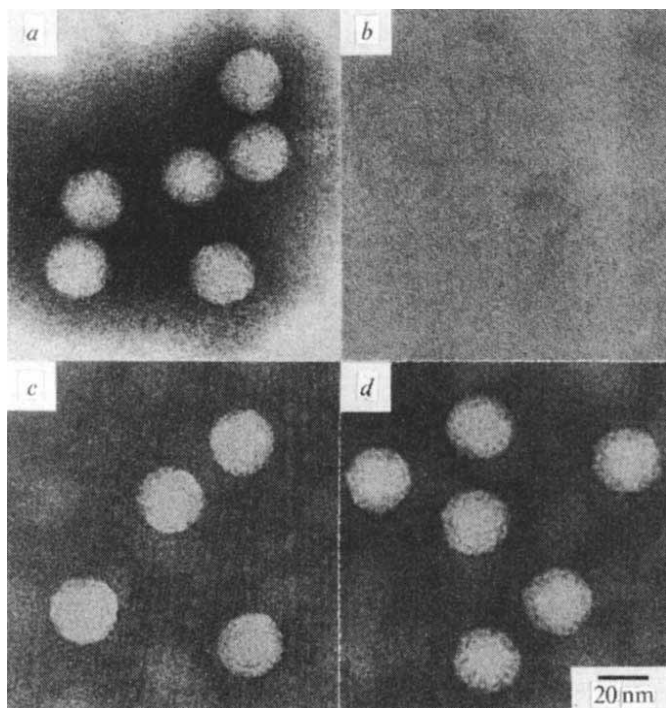


Fig. 2 Electron micrographs of FMD virus and swine vesicular disease virus before and after treatment with carbon dioxide. Droplets containing virus were placed on electron microscope grids and exposed to either moist CO_2 gas or moist air for up to 10 min. After draining they were stained with phosphotungstic acid at pH 7.2. — *a*, FMD virus; *b*, FMD virus plus CO_2 ; *c*, swine vesicular disease virus; *d*, swine vesicular disease virus plus CO_2 .

Rabbits inoculated intravenously, and guinea-pigs intradermally in the plantar pads with the 1972 England virus showed no evidence of virus multiplication or of lesions. I.p. inoculation of 6-day-old mice with $10^{4.5}$ TCID₅₀ of virus however caused paralysis and death between 3 and 8 days after inoculation.

Samples of vesicular fluid and an extract of vesicular epithelium from two field cases were each inoculated intradermally into the tongue of one steer and the bulbs of the heels of two pigs. No lesions were observed in the cattle, but in the pigs, lesions became apparent after 24 h when there was blanching of the epithelium around a number of the inoculation sites. After a further 12 h, vesicles had appeared on the heel bulbs

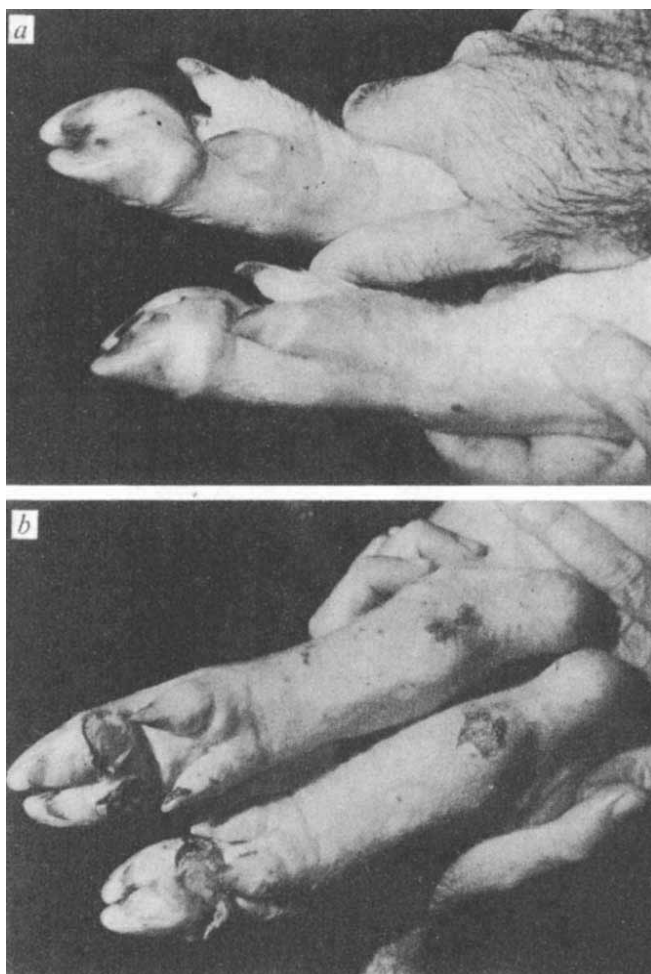


Fig. 3 Feet of pigs infected with the swine vesicular disease virus (England 1972) showing lesions (*a*) 1 day old and (*b*) 8 days old.

and later all feet became involved with further vesicles developing in the interdigital area and around the coronary bands of the primary and accessory digits (Fig. 3). Vesicles and erosions were observed on the lips, gums and tongue of some pigs, and fever (up to 42°C) was noted. Samples of vesicular epithelium from the pigs, tested by complement fixation, gave positive results with the Italy 1966 enterovirus antiserum and yielded up to $10^{7.6}$ TCID₅₀ of virus per gram of epithelium when titrated on IB-RS-2 cell monolayers.

Two days after inoculation of the four pigs, a further eight were placed in contact with them. All developed generalized disease of a similar description after 2 to 6 days and neutralizing antibody to the England 1972 virus was demonstrable in their serum after 4 days (R. S. Hedger, personal communication).

It seems that the causative agent of these first recorded British outbreaks of swine vesicular disease is a porcine enterovirus, serologically similar to those responsible for the Italian and Hong Kong outbreaks. The generally slower spread and lower morbidity observed in the field, when compared with FMD, may be explained by a lower output of virus from infected pigs. To obtain an estimate of the virus content of the animals' environment, pens containing infected pigs were sampled for the presence of virus. Slurry collected from the floor yielded up to 10 TCID₅₀ per ml. during the period that pigs showed clinical lesions. Air sampled by collecting swabs of nasal mucus from personnel working in the pens during the same period contained no recoverable virus. These results suggest that the amount of viral excretion from the pigs was considerably less than would occur with pigs infected with

FMD, and further work is in progress to investigate methods of spread.

The origin of the virus is at present subject to speculation. There are recent reports from Europe of several similar outbreaks in pigs. Samples from infected pigs sent to Pirbright from outbreaks in two other countries have been tested by complement fixation and given positive results with the Italy 1966 antiserum. Further work is being carried out to establish the serological relationship of the England 1972 virus to porcine enteroviruses isolated from concurrent and past outbreaks of vesicular disease in pigs.

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Evidence for an Empty Core in a Bacterial Flagellum

BACTERIAL flagella have three parts; a basal structure which is attached to the plasma membrane, a hook-like structure and a long filament which is outside the cell envelope. Flagellar filaments have been extensively studied¹ and have been shown to be composed of an array of flagellin subunits. Various models have been proposed for the arrangement of the subunits in the flagellum, a common feature of which is a helical array with a hollow central core. Evidence for such a core has only rarely been obtained in electron micrographs of negatively-stained flagella when the flagella are still attached to the bacterium², although in the proposed models the core is sufficiently large to allow easy penetration of negative-staining solutions.

Flagellar filaments grow at the end distal to the bacterium, and flagellin is synthesized on ribosomes within the cell; these observations therefore raise the question of how the flagellin molecules are transported to the site of assembly. Kerridge¹ considered that it is most improbable that flagellin molecules are excreted into the growth medium for subsequent assembly onto flagellar filaments, or that they travel along the outside of the flagellum, and concluded that the molecules enter the basal structure and then travel through the centre of the filament to the distal end. If this is correct, it would explain why negative staining solutions are unable to penetrate into the core of the flagellum, as the core would be blocked by flagellin molecules. There is no direct evidence, however, for the presence of free flagellin within the flagellum.

We were therefore interested to see apparently empty cores in flagella in freeze-etched preparations. Frozen cultures of the hyperthermophilic bacterium, *Clostridium thermohydrosulfuricum*, strain L77³, in the late logarithmic phase of growth, were fractured at -196°C ^{4,5}. Figs. 1 to 3 illustrate flagella lying on the surface layer of the cell wall which is composed of a hexagonal array of subunits.

Different fracture planes are seen in the flagella, and in some regions part of the flagellum has broken away to reveal an

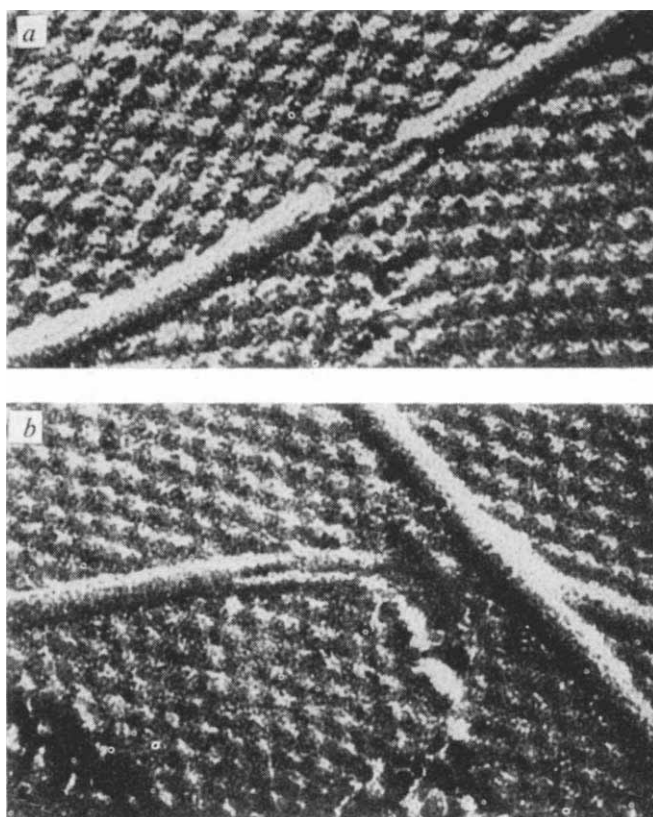


Fig. 1 *a* and *b*, Flagella about 15 nm in diameter lying on the surface of the cell wall. The regular array of subunits on the cell wall is partly destroyed near the fractured regions of the flagella. $\times 120,000$.

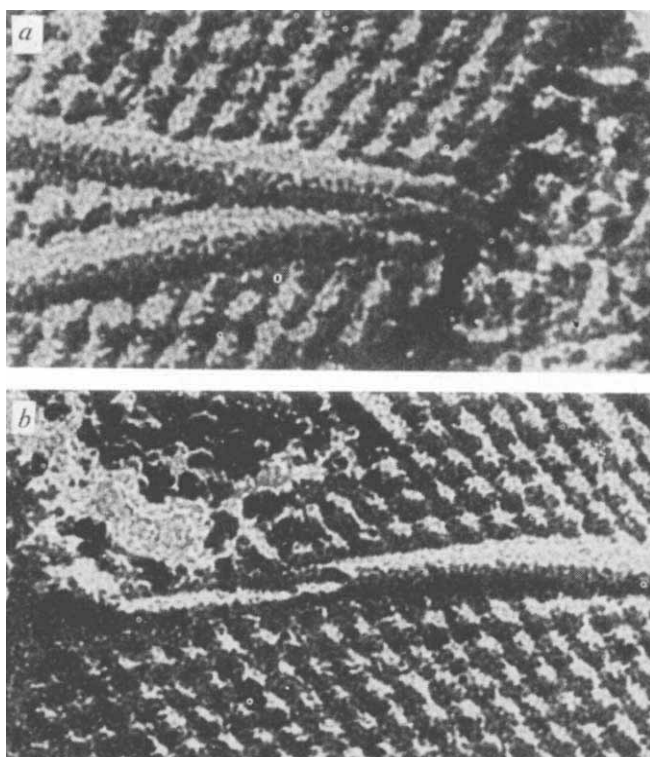


Fig. 2 *a* and *b*, An empty core is visible in cross-fractured flagella in regions where the cell wall and cytoplasmic membrane have also been split away. *a*, $\times 200,000$; *b*, $\times 140,000$.

empty core. An examination of the micrographs makes it clear that these cores are revealed by the freeze-fracturing and etching process, and are not errors in flagella synthesis. Near

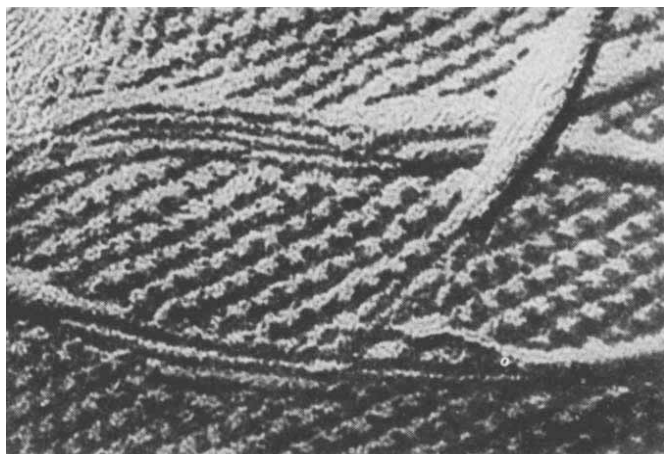


Fig. 3 A group of flagella, two of which are lying parallel to each other, all show longitudinal fractures and hollow cores. $\times 120,000$.

the sites along the flagella, where the core is visible, the regular pattern on the surface of the cell wall is sometimes partly destroyed (Fig. 1a and b) indicating that these are regions of fracture. The majority of the flagella which show the cores are fractured in regions in which parts of the wall and cytoplasmic membrane have also been split away (Fig. 2a and b). The fact that the fractured flagella always appear as hollow half-cylinders is evidence that they were originally filled with etchable material. If densely-packed flagellin molecules had been present there would be an equal probability that they would either remain within the fractured regions and be seen protruding from the half-cylinders, or be removed with the half of the flagellum. Complete confirmation of this can be obtained by the study of complementary fracture faces⁴.

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Alpha Rhythms and Hyperkinesia

IN his comment on my report "Alpha Rhythms in the Hyperkinetic Child"¹, Davidson² stated that my data support Lippold's hypothesis³ that "Oscillation in striate muscle control systems is the source of alpha rhythm". He quoted the work of Mulholland and Evans^{4,5} which suggested that presence or absence of alpha rhythms was not determined by the presence or absence of relaxed wakefulness, but by oculomotor function. Many arguments have been presented against such a hypothesis⁶⁻⁹. Butler and Glass¹⁰ have presented findings inconsistent in a number of respects with the view of ocular tremor as a generator of alpha rhythm, and suggested that eye movements in their turn may be modulated by alpha rhythms when conscious control of fixation waned or was interrupted by eye closure. Chapman *et al.*¹¹ repeated the basic experiments of Mulholland and Evans^{4,5}, taking care to eliminate effects of

differential visual input, and demonstrated that alpha activity was influenced by visual input and not by eye position.

Contrary to what Davidson states², the article by Darrow *et al.*¹² does present data from a simplistic experiment showing that alpha returns with "automation" or habituation to a stimulus. Dempsey and Morrison's¹³ concept of a sub-cortical thalamic pacemaker, Jasper's¹⁴ work describing an intrathalamic pacemaking system, the theory of Andersen and Andersson involving recurrent inhibitory systems in the thalamus and the relation of all these to human alpha rhythms has been reviewed by Andersen and Andersson¹⁵. Barlow and Estrin¹⁶ have recently presented evidence which suggested that alpha activity represented summated polysynaptic potentials under thalamocortical control.

Habituation of the alpha attenuation response has been studied in hyperkinetic children, and exaggerated initial response to novel stimuli and decreased capacity to disattend to redundant events has been demonstrated¹⁷. In a study of the alpha average, greater spread of frequency abnormalities and poor spatial organization has also been shown in such children¹⁸. In a current prospective study I have observed that about 60% of hyperkinetic children do not have well organized alpha rhythms in amounts appropriate for their age. Preliminary observations seem to indicate that with increasing age and decreasing hyperkinesia, alpha activity shows proportionate increase in amount and organization in these children. These observations in addition to the literature cited above lend some support to the contention that alpha rhythm in the human EEG is an index of relaxed wakefulness.

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Amino-acids in Nectar and their Evolutionary Significance

IT has been known since ancient times that nectar, usually secreted from specialized glands within flowers, is utilized by anthophilous (flower-visiting) animals for the energy-providing sugars that it contains. It is usually assumed that protein-making materials will be obtained elsewhere, from

larval feeding (Lepidoptera), from pollen (Hymenoptera—Apioideae) or (by vertebrates) from insects. However, some butterflies are known to be attracted to decaying flesh, faeces, urine and stagnant water¹ and well as sweat², while many take phloem-sap from a wound or honey-dew excreted by aphids³. Moths are known to drink fruit juices, sweat, secretions from the eyes of animals, and even mammalian blood⁴. Unusual feeding-behaviour is shown by butterflies of the genus *Heliconius* which collect pollen, steep it in nectar, and subsequently ingest the amino-acids that diffuse from the grains⁵.

This evidence of concern with amino-acid ingestion by adult insects usually thought of as restricted to a liquid diet suggests that nectar itself should be examined as a potential source. Some comprehensive analyses of honey (which contains additives provided by its makers, the honey bees), have revealed the presence of substances other than carbohydrates⁶⁻¹⁰, but analyses of nectar^{11,12} appear to have been virtually restricted to studies of the amounts and proportions of the sugars present. Consequently, a survey of 266 species of flowering plant occurring in nature in California and in the University of California Botanical Gardens has been made. The results show that the occurrence of significant concentrations of amino-acids in nectar is the rule.

Nectar was taken from flowers at the time of maximum production, usually mid-morning, using a fine capillary tube, taking precautions to avoid any contamination by pollen. Nectar drops placed on filter-paper were allowed to dry and then stained with ninhydrin (0.2% in acetone) and up to 24 h at laboratory temperature allowed for the usually violet colour to come to a maximum. A comparison scale for colour depth was created by making dilutions of an aqueous solution of histidine and staining these (1=49 μ M; 2=98 μ M;; 10=25 mM). Each sample was scored again this linear "histidine scale". Proteins (tested electrophoretically in several samples) seem not to be present in quantities significant for animal nutrition.

Table 1 Amino-acid Concentration of Nectar in Flowers of Different Pollination Type

Flower pollination type	No. of species	Mean (on histidine scale)	Range	Coefficient of variation (%)
Bee	95	4.76	0-9	47
Butterfly and bee	44	6.02	3-8	23
Butterfly	41	6.68	4-10	21
Moth	30	5.60	0-9	45
Bird	49	5.22	1-9	37
Fly (unspecialized)	34	3.77	0-9	57
Fly (specialized)	8	9.25	7-10	—

A summary of results on a pollination type basis is given in Table 1. The nectar richest in amino-acids is that which is produced in small quantity by flowers that by their appearance and smell attract carrion- and dung-flies away from their usual sources of nutrients. Nectars produced rather copiously by flowers which are primarily visited by butterflies are also consistently rich in amino-acids, slightly more so, on the average, than those from flowers visited freely by both butterflies and bees. Judging by their mean score on the "histidine scale" (6.68), about 0.3 ml. of "butterfly flower" nectars would contain about 840 nmol of amino-acids. This is the quantity of free amino-acids that Gilbert⁴ believes would be obtained from a day's harvest of pollen (1.5 mg) by the uniquely pollen-collecting *Heliconius* butterflies (apparently producing a profound effect on their life-span and reproductive output). 0.3 ml. of nectar could be provided by no more than fifteen flowers of *Dianthus barbatus*, and some "butterfly flowers" produce more than this species. Consequently, nectar cannot be ignored as a potential source of amino-acids for the nutrition of butterflies as well as carrion- and dung-flies.

On average, the amino-acid concentration in the nectars of flowers visited by bees is significantly lower than that of "butterfly flowers", even though less is generally produced. Values range from 0 to 9 on the "histidine scale". These results suggest that for bees, which have a rich alternative source of proteinaceous materials in pollen, the amino-acids in nectar are less important than they are for butterflies. An even lower mean is shown by "unspecialized fly flowers" that expose their nectar freely to syrphids and other diptera (as well as bees and beetles) that also taken pollen as food. In 1873 Hermann Müller⁵ described and interpreted correctly the behaviour of the dipteran *Rhingia rostrata* taking both nectar and pollen from the same flower of *Lythrum salicaria*.

For moths, there is also a wide range of amino-acid concentrations in the nectars of the flowers they prefer. The same is true of "bird flowers" (where a rather low mean is explicable on the basis of the birds' utilization of an alternative protein source in insects).

Flowering plants belonging to some taxa notable for "primitive" characters (such as *Drimys winteri*, *Liriodendron tulipifera*, members of the Ranunculaceae, and some palms) contain some amino-acids in their nectar. Consequently, it may be supposed that amino-acids have been a constituent of flowering-plant nectars since the early stages of their evolution. Nevertheless, there is evidence that, as the evolution of specialized pollination systems has proceeded, selection has taken place for consistent high concentrations of amino-acids. No satisfactory linear phylogenetic arrangement of flowering plant families exists; nevertheless data from the available taxa may be subjected to a series of comparisons based on their possession of what are generally considered "primitive" or "advanced" characters, taken one at a time (Table 2). Consistently, the means for plants showing the "advanced" character are higher (while the coefficient of variation is smaller). However, there are differences with a direct taxonomic basis; high values were given by the tested members of such families as the Asclepiadaceae, Liliaceae, Campanulaceae and Leguminosae, while notably low values characterize the tested members of the Myrtaceae, Caprifoliaceae and Saxifragaceae. The Rosaceae shows a wide range of values.

Table 2 Amino-acid Concentration in Nectar in Different Flower Types

	No. of species	Mean (on histidine scale)	Coefficient of variation (%)	P*
Polypetalous	105	4.75	45	<0.001
Sympetalous	161	5.90	34	
Actinomorphic	158	5.15	42	=0.001
Bilateral	108	5.99	34	
Exposed nectar	73	4.88	48	=0.01
Concealed nectar	193	5.66	36	
Many stamens	47	4.51	51	<0.005
Few stamens	219	5.66	37	
Polycarpous	24	4.88	39	=0.10
Syncarpous	292	5.54	39	
Hypo- and perigynous	168	5.29	42	<0.60
Epigynous	98	5.46	37	

* Probability that difference between means is due to chance.

Chromatographic analyses of the nectars of forty-four of the species show that a wide range of different amino-acids is to be found, with all essential amino-acids¹³ available from the nectar of one species or another. The nectar of *Dianthus barbatus* (Caryophyllaceae), a "butterfly flower", contains at least twelve different amino-acids, the largest number yet found in a single species. Consequently, at least supplemental nutritional value for the flower-visitors can be attributed to the amino-acids of the nectar. Their presence and the apparent selection for their increasing concentration in nectar raise many interesting questions as

to the evolutionary mechanisms involved. A detailed report on these continuing studies will be presented elsewhere¹⁴.

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The Pathogenicity of Nodamura Virus for Insects

NODAMURA virus, which was originally isolated from mosquitoes from Nodamura, near Tokyo, multiplies in several insect and tick species, apparently without causing symptoms. It is transmissible to suckling mice by infected *Aedes aegypti*, and is fatal¹. In Japan young pigs have been found with antibodies to the Nodamura virus, which has been classified as an unusual kind of arbovirus because it is resistant to ether². This property, together with other features of the virus particles³ are reminiscent of acute bee-paralysis virus⁴ and we therefore examined its effect on the honey-bee, *Apis mellifera mellonella*.

A 2% suspension in 0.01 M phosphate buffer, pH 7.0, of homogenized brain tissue from mice killed by Nodamura virus was emulsified with carbon tetrachloride and the supernatant fluid cleared by low speed centrifugation. Adult bees, each injected with 1.0 µl. of the preparation, died after a minimum incubation period of 7 day at 30° or 35° C. Their anterior two pairs of legs became paralysed a few hours before death. The virus was serially passaged in bees, and an extract containing the equivalent of 10⁻⁷ of a bee killed by Nodamura virus was infective by injection into healthy bees. Isometric particles, about 30 nm in diameter, identical in shape and size to those seen in muscle tissue of mice killed by the virus³ were purified by triturating infected bees in phosphate buffer with a quarter volume of ethyl ether, emulsifying the mixture with carbon tetrachloride and then subjecting the aqueous phase to cycles of high and low speed centrifugation⁵. Virus preparations reacted strongly in Ouchterlony gel-diffusion tests with antiserum prepared against Nodamura virus and formed single precipitin lines confluent with those formed by the original mouse brain inoculum. Antiserum from rabbits we injected with virus from bees also gave a single homologous precipitin line against virus from bees and mice. Nodamura virus did not react with antiserum to acute bee-paralysis virus. Wax moth larvae injected with an extract containing the equivalent of 10⁻³ of a bee killed by Nodamura virus died, mostly as pupae, after 7–14 day at 30° C. A crude extract of each of these pupae in 1.0 ml. water with 0.2 ml. ether gave an intense

single precipitin line, confluent with Nodamura virus from bees, in gel-diffusion tests.

Our results indicate that Nodamura virus is a virulent insect virus of a kind which, contrary to widely held opinions about insect pathogens, can multiply in and kill mammals.

We thank Dr F. A. Murphy for supplying brain tissue of mice killed by Nodamura virus, Professor W. F. Scherer for supplying antiserum, and Dr K. S. Kim for electron microscopy.

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Nature of the Lepidopteron Sensing Mechanism: Possible Photochemical Response

THE complex nature of the moth sensing problem has been demonstrated^{1–4}. Here we suggest that the interaction of a moth with its radiation environment may be explained by a chemical change in metabolism induced by the radiation. We have tested this hypothesis by studying the temperature dependence of the behavioural response of several moth species to a black-light source and coherent submillimetre wave radiation.

Behavioural experiments were carried out with equipment previously described⁵, in which moths were exposed to uniform radiation in a test chamber. Experimentally, the response of an individual moth was indicated by its attraction to the end of the test chamber at which the radiation source was located.

If the radiation source induced altered chemical metabolism mediated by change of photochemical behaviour, an individual moth would not respond to the radiation stimulus until the chemical reaction had been triggered. Assuming that the moth response follows a first order law, and if C_m is the concentration of moths in the test chamber which have not responded to the radiation, then

$$\frac{dC_m}{dt} = -k C_m \quad (1)$$

where k is the rate constant. That is,

$$\ln(C_m) = -kt + k' \quad (2)$$

where k' is a constant of integration.

In our experiment with black-light, the source was a 'General Electric F15T8-BL' lamp, having a fluorescent band with a peak in the ultraviolet (below 0.38 µm). The broad fluorescence ended at 0.45 µm and had mercury peaks in its spectrum. Some emission was observed around 8 µm, but this was no greater than a black body at 45° C. There were no significant bands beyond 0.45 µm.

It was found that the response of both the almond moth, *Carda cautella*, and the fall armyworm, *Spodoptera frugiperda*, to the BL source followed first order kinetics. The response of

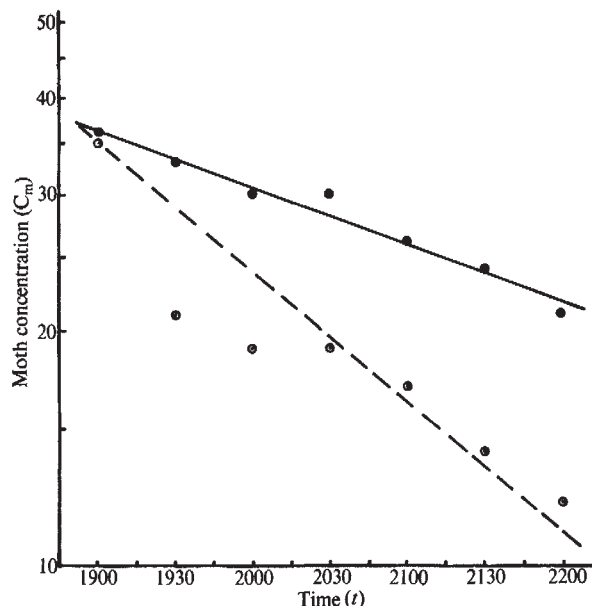


Fig. 1 Response rates of male *S. frugiperda* to a BL source. ●—●, At 20.8°C; ○---○, at 23.9°C.

male *S. frugiperda* to a 4 W BL source at 20.8 and 23.9°C (Fig. 1) is in agreement with equation (2). We suggest that first order chemical reactions may be involved in the sensing responses.

The rate of response of male and female *S. frugiperda* to a BL source follows the Arrhenius equation (Fig. 2)

$$k = A e^{-E/RT} \quad (3)$$

where A is a constant, T the absolute temperature, R the gas constant and E is the activation energy for the process. A value of 47×10^3 cal mol⁻¹ for the activation energy was deduced from Fig. 2. This value is high although not outside the range of normal biochemical reactions.

The same pattern of behaviour was also observed with several other species at different wavelengths with differing rate constants. It appears therefore that this type of response is not uniquely associated with one species of moth or one radiation source. The response of the male corn earworm, *Heliothis zea*, to 311 μm (16 mW) and 128 μm (20 mW) and the male *S. frugiperda* to 311 μm (16 mW) is shown in Fig. 3a, as well as the response of male *S. frugiperda* to 7.5 W BL source for comparison. The response rates to coherent submillimetre wave radiation are much higher than that to the BL source, at a fixed temperature the response rate is a function of the radiation wavelength.

The response of male *H. zea* and *S. frugiperda* to 337 μm (50 mW) radiation is shown in Fig. 3b, both species show their peak response at this wavelength³. The behaviour patterns form two distinct lines with different slopes. The first is triggered rapidly as the radiation source is turned on and ceases after a period of 10 to 15 min; the second has a lower rate constant and persists longer.

This biphasic response pattern could be explained as a 337 μm radiation induced metabolic shift from a rapidly utilized metabolic source to one used more slowly. Based on work by Sacktor and Wormser-Shavit⁵ and of other authors^{6,7} we believe that the important metabolites involved in this type of biphasic response are glycogen and proline. We suggest that glucose catabolism is greatly stimulated at 337 μm by the release of a neuroendocrine factor such as a catecholamine from the corpus cardiacum⁸. After the first 10 to 12 min of laser exposure, glucose catabolism shuts down and proline catabolized,

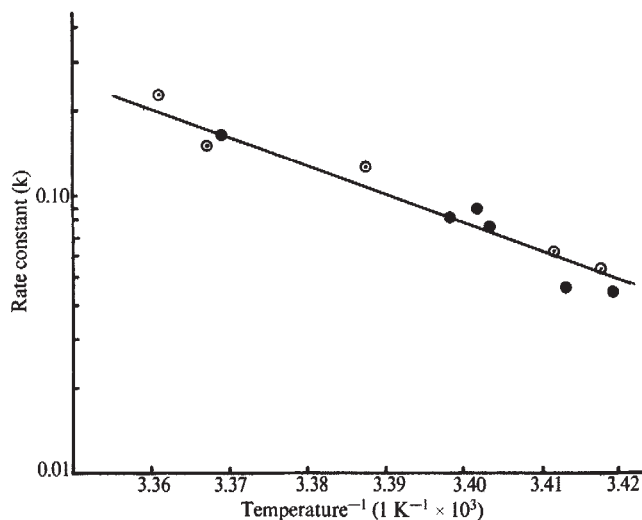


Fig. 2 Response rate constant as a function of temperature. *S. frugiperda* subjected to a BL source. ○, Female; ●, male.

corresponding to the lower response rate in Fig. 3b. The increased rate of glycogen breakdown as shown by accelerated respiratory rates under 337 μm stimulation, is quickly replaced by proline utilization in the Krebs cycle. Unfortunately, the role of proline in the respiration of Lepidoptera has not been fully resolved.

Three factors showing radiation-induced physiological alterations are apparent. First, there is a pronounced stimulation

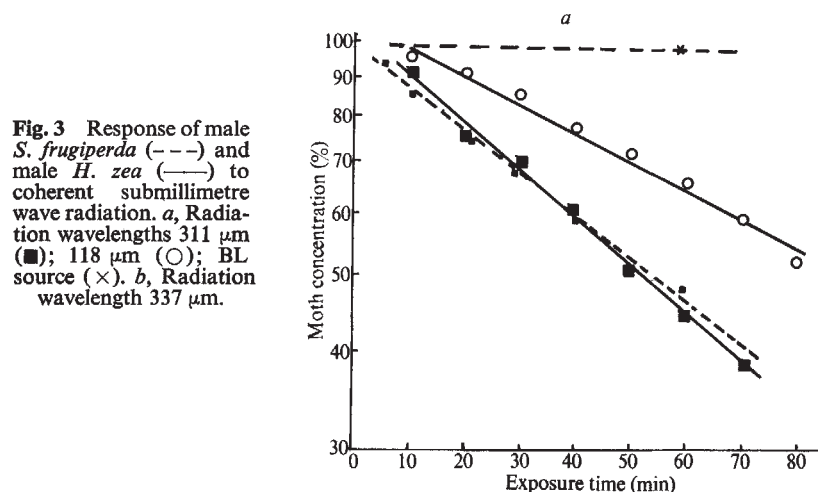
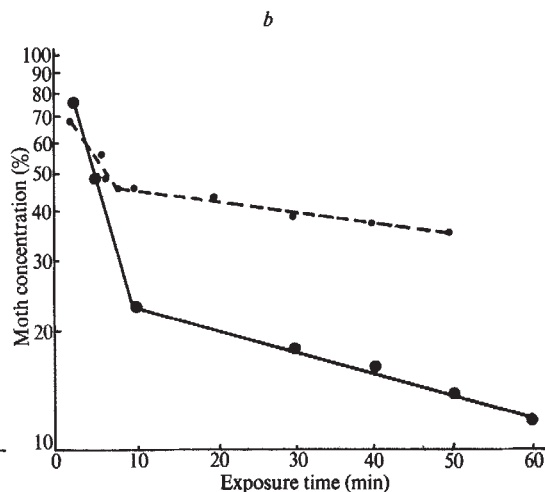


Fig. 3 Response of male *S. frugiperda* (---) and male *H. zea* (—) to coherent submillimetre wave radiation. a, Radiation wavelengths 311 μm (■); 118 μm (○); BL source (×). b, Radiation wavelength 337 μm.



of respiration by a black-light source. Second, the response to black-light is considerably altered by pre-exposure of moths to 0.6328 μm radiation from a helium-neon laser. Third, irradiating moths with 337 μm radiation inhibits activity and mating potential. We suggest that a chemical process, possibly an enzyme system, is triggered and rate adjusted by radiation exposure.

It is apparent that further studies are needed to increase our understanding of these radiation induced behavioural responses. If stimulation of respiration is triggered photochemically, knowledge of the kinetics involved could result in a method of radiation exposure to stimulate a high percentage of the moth population to the high respiration output. This high output would cause the moths to "burn themselves out" and reduce their life cycle and mating potential, and could be of value as a means of practical control.

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Early Occurrence of Domestic Sheep in Sub-Saharan Africa

RECENT excavations by the South African Museum at a coastal cave situated at Die Kelders some 100 miles east of Cape Town have revealed the earliest definite sheep remains in Sub-Saharan Africa.

A minimum of twenty-three individuals were represented and came from a well defined shell midden (layer 2 a/b), two from above and one from immediately below this layer.

A total of nineteen mandibles and maxillae of sheep have been grouped into age categories based on the wear and stage of tooth eruption^{1,2}. The results, which are obviously very approximate, indicated the following clustering; four were less than two months old, four considerably older than 40 months (the approximate age at which the last molar erupts), and the remaining 15 were between six to thirty months.

There was good bone preservation because of the alkaline nature of the deposits which underlie the limestone cave roof, and although dentition was counted, other parts were also represented. Sheep and goats are most readily differentiated by the nature of their occipits and horn cores and four of each of these, which definitely belonged to sheep, were represented. Identification was further supported by early reports from the Cape which frequently mentioned sheep but never the presence of goats. These sheep, according to a number of sources³ (the earliest being Vasco da Gama in 1497), were hairy, non-wool bearing, relatively large and characterized by their broad, fat tails.

A number of radio carbon dates, all from charcoal fragments, have been obtained from this layer. These are GX 1685, 1465 $\pm$ 100 years BP = AD 485⁴, GaK 3877, 1650 $\pm$ 90 years BP = AD 300, GaK 3878, 2620 $\pm$ 90 years = 670 BC, GaK 3955, 1600 $\pm$ 120 years = AD 350. There is a date of GaK 3956,

1590 $\pm$ 80 years BP = AD 360 for the midden (3) immediately below (2 a/b).

The inconsistency of the 670 BC date may be because samples GaK 3877 and GaK 3878 were not collected *in situ*; in GaK 3878 had been obtained from layer (2 a/b) in a square not fully covered by this layer. The older date is at present considered unreliable.

Comparison of the fauna in the sheep-containing midden with that of the two underlying shell middens which lacked sheep, showed no significant differences either in the relative frequencies or in the condition of the bones. The sheep mandibles, with the exception of a few very young individuals, are characterized by having damaged inferior margins, a feature not observed in the case of small antelope mandibles. Small antelope teeth show no age clustering and individuals of all ages ranging from new-born to extreme old age were present.

Differences in the cultural material were more marked. Layer (2 a/b) had forty-two bone implements, whereas the lower two middens combined had thirty with a 30% greater bulk of excavated material. Midden (2 a/b) contained both decorated incised bone points, a bone "spoon" as well as utilized sheep and bovid scapulae, none of which were found in the underlying middens. In addition it contained eleven bone spatulas known from historical sources to have been used to dehair skin⁵ as opposed to only one in midden (4) and none in midden (3). The pottery of (2 a/b) differs in that it includes a decorated pot as well as a spout shard quite unlike the potsherds of other middens. Other finds unique to (2a/b) included perforated *Phalium zeylonica* shells and an unusual piece of mastic which apparently belonged to a four-pronged arrow.

The absence of any locally occurring indigenous sheep, coupled with the known presence of domestic sheep in East Africa circa 850 BC⁶ suggested that the Die Kelders sheep were a domesticated variety. Differences in cultural material with more sophisticated decorations and greater use of bone suggests, at least, a contact with people other than those responsible for the deposition of the lower middens.

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Radiosensitivity of the Liverwort *Marchantia polymorpha* L. to Internal Radioisotopes

THERE are very few reports concerned with the effects of internal radioisotopes on the growth of plants. The growth of lateral roots of *Zea mays* is stopped with the addition of 10 $\mu\text{Ci ml}^{-1}$ of ³H-thymidine¹, whereas generally the growth of plants as a whole is not disturbed by doses of radioisotopes normally used for biochemical studies, even though injury, or even growth stimulation may occur in some cases².

We present here evidence of very great sensitivity of young plants of *Marchantia polymorpha* to internal radioisotopes ³²P

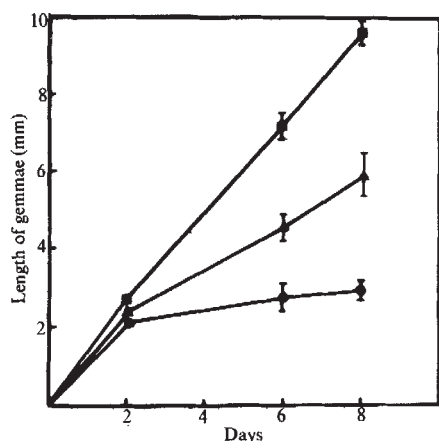


Fig. 1 Effect of internal ^{32}P on the growth of *Marchantia polymorpha*. ■, Control; △, $5 \mu\text{Ci ml}^{-1}$; ●, $10 \mu\text{Ci ml}^{-1}$.

and ^{35}S , at doses generally used for labelling. Twenty to twenty-five gemmae from gemmae cups of female *Marchantia polymorpha* were cultivated in Petri dishes on 15 ml. of nutrient solution solidified with agar as indicated by Schneider *et al.*³. The growth conditions were continuous illumination at 1,500 lx and a temperature of 22°C . Carrier-free $^{32}\text{P-H}_3\text{PO}_4$ (CEA, Saclay, France) in 0.01 M HCl solution was added to the nutrient medium, and the same amount of HCl was added to the controls.

At a dose of $5 \mu\text{Ci ml}^{-1}$ of ^{32}P (specific activity: $0.4 \mu\text{Ci } \mu\text{g}^{-1}$ of P) the growth of thalli was seriously inhibited and at $10 \mu\text{Ci ml}^{-1}$ it was stopped. The inhibiting effect was measurable after 48 h of growth and the difference between control and treated plants was significant after about 3 days of culture. All thalli treated with the radioisotopes were affected and growth inhibition was visible on both length and surface of the thalli. This experiment was repeated several times and the same response was always observed. At doses that did not completely inhibit growth (3 to $8 \mu\text{Ci ml}^{-1}$) all portions of thalli were uniformly affected.

When carrier-free ^{35}S was used instead of ^{32}P , inhibition of growth was complete for $80 \mu\text{Ci ml}^{-1}$ ($0.76 \mu\text{Ci } \mu\text{g}^{-1}$ of S). As the energy of β particles emitted by ^{35}S is about the tenth of the energy of β particles emitted by ^{32}P we concluded that comparable inhibiting effects were obtained for equal flux of energy. Therefore the nature of the element is not by itself involved in the observed inhibition.

The effect of acute γ -irradiation on *Marchantia* has been investigated by Miller and Sparrow⁴. Contrary to our results with internal radioisotopes they found that the radiosensitivity of vegetative non apical cells was comparable to that of higher plants having chromosomes of similar size. Apical cells possess bigger chromosomes and are more radiosensitive to γ -irradiation. Since entire thalli are uniformly affected by internal radioisotopes, their action could not be the result of damaging chromosomes in the more sensitive apical cells.

It is certainly worth remembering before further studies that thalli of *Marchantia* are haploid and could therefore be more radiosensitive than diploid plants.

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Geographic Differences in Phytoplankton Sensitivity to PCBs

DIFFERENTIAL sensitivity of marine phytoplankton species to chlorinated hydrocarbons has been demonstrated in pure^{1,2} and mixed³ cultures. In particular, the toxicities of DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) and PCBs (polychlorinated biphenyls), both widespread pollutants in the marine environment⁴, have been investigated. Evidence does not exist, however, for correlating organochlorine sensitivity with the ecological adaptations of the organisms tested.

Previous reports suggest the existence of physiological races of marine phytoplankton. Guillard and Ryther⁵ showed *Thalassiosira pseudonana* from the Sargasso Sea (clone 13-1) to be stenothermal and stenohaline, unlike the continental shelf and estuarine isolates (clones 7-15 and 3H). Intraspecific differences in half-saturation constants (K_s) for nutrient uptake have also been demonstrated; isolates from the low-nutrient waters of the Sargasso Sea had lower K_s values for nitrate⁶ and silicon⁷ than did coastal clones. Such findings indicate that races of phytoplankton are best adapted to their respective environments. Slobodkin and Sanders⁸ have postulated that organisms adapted to stable, "predictable" environments would, in general, be more sensitive to environmental stress than would organisms adapted to "unpredictable" surroundings. Experimental evidence for this hypothesis is lacking. We therefore investigated the toxicity of PCBs to three species of diatoms, comparing clones isolated from the relatively stable Sargasso Sea waters⁹ with those from fluctuating estuarine environments.

The diatoms tested were *Thalassiosira pseudonana*, *Fragilaria pinnata*, and *Bellerophia* sp.; Fig. 1 presents the clones studied and their isolation sites. (For exact information on dates and sites of isolation, see Carpenter and Guillard⁶.) Cells were grown axenically in f/2 medium⁵ prepared from Vineyard Sound seawater (salinity = 33‰) filtered through a precleaned Amberlite XAD-2 resin to remove any chlorinated

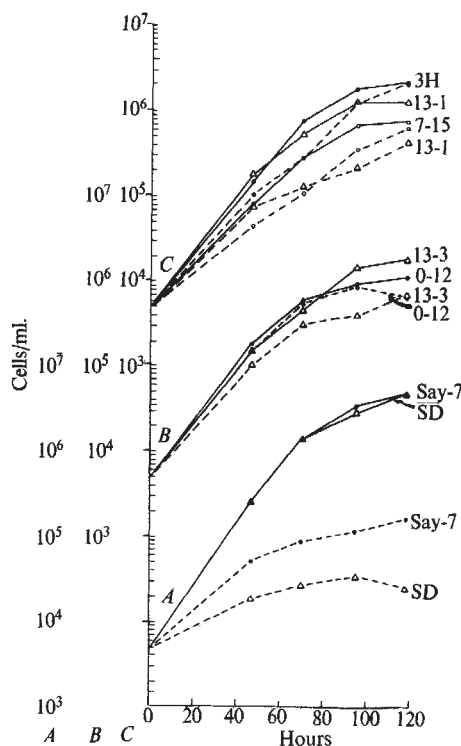


Fig. 1 Growth of (A) *Bellerophia* sp., (B) *Fragilaria pinnata*, and (C) *Thalassiosira pseudonana*. Clones 13-1, 13-3, and SD were isolated from the Sargasso Sea; 7-15 from the edge of the continental shelf; 3H from Moriches Bay, Long Island; 0-12 from Oyster Pond, Massachusetts; and Say-7 from Great South Bay, Long Island. Data points are the means of three replicates. —, 0 p.p.b.; ---, 10 p.p.b.

Table 1 Ratios of Mean Cell Densities from Treated : Control Cultures at Five Sample Times

	0	Hours after inoculation			
		47.5	70	95	118
<i>T. pseudonana</i> clones					
3H (estuarine)	1.00	0.694	0.365	0.676	0.976
7-15 (estuarine)	1.00	0.544	0.368	0.520	0.844
13-1 (oceanic)	1.00	0.422	0.241	0.172	0.341
<i>F. pinnata</i> clones					
0-12 (estuarine)	1.00	0.824	0.909	0.932	0.590
13-3 (oceanic)	1.00	0.688	0.707	0.269	0.387
<i>Bellerophia</i> clones					
Say-7 (estuarine)	1.00	0.207	0.064	0.036	0.035
SD (oceanic)	1.00	0.074	0.020	0.013	0.005

hydrocarbon^{9a} contamination. Exponentially growing cells were inoculated into sterile, tightly sealed screw cap bottles containing 150 ml. of medium to give an initial cell density of 5,000/ml. The procedure for adding PCBs ('Aroclor 1254') has been described². For each clone, there were three cultures containing 10 parts per 10⁹ (p.p.b.) of PCBs and three controls without PCBs. The cultures were maintained at 20° C and illuminated 14 h/day with 5,800 lumens/m² from cool-white fluorescent bulbs. Samples of 1 ml. were fixed with 0.05 ml. of Lugol's solution and counted with a Speirs-Levy Eosinophil counter.

Although growth of all seven clones diminished with PCB treatment (Fig. 1 A-C), isolates from the Sargasso Sea invariably were more sensitive than were clones from estuaries and the continental shelf (Table 1). Clone 7-15, from the continental shelf, was, in turn, more sensitive than clone 3H, its counterpart from the Moriches Bay estuary. Both *Bellerophia* clones were more sensitive than all others tested, but here, too, oceanic clone SD proved more sensitive than did estuarine clone Say-7. Two-way analyses of variance¹⁰ on the logarithmically transformed data showed that, at 95 h, the interaction term between PCB treatment and geographic origin was significant for *T. pseudonana* ($F(2, 12) = 34.56$, $P < 0.001$), for *F. pinnata* ($F(1, 8) = 31.57$, $P < 0.001$), and for *Bellerophia* sp. ($F(1, 8) = 12.81$, $P < 0.01$), indicating algal sensitivity was highly dependent on the sites of isolation.

Natural waters generally contain somewhat lower concentrations of PCBs (one to three orders of magnitude lower) than we employed in our experiments (ref. 11 and unpublished results of G. R. Harvey, W. G. Steinhauer and J. M. Teal), although concentrations exceeding ours have been detected¹². Concentrations in water may be misleading, however. Organisms are probably exposed to greater amounts of chlorinated hydrocarbons than concentrations in natural waters would indicate because these substances are rapidly removed from water by organisms, including phytoplankton¹³. Holden¹⁴, for example, found no PCBs in Scottish waters but substantial amounts in the resident organisms.

Our results suggest that estuarine-coastal phytoplankton may be better equipped to survive abiotic environmental stress than are those found in the open ocean. These data are therefore consistent with the hypothesis of Slobodkin and Sanders⁸.

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Genetic Analysis of Tail Rattling in the Mouse

TAIL rattling is a prominent feature of aggressive encounters between mice and is thought to be a threat behaviour, although conclusive evidence of this is lacking¹. A factor analysis of aggressive behaviours elicited in a single subject using footshock in conjunction with a bottle brush target indicated that tail rattling had a high loading on the "aggressive factor" and negligible loading on the other factors². On the other hand, a rather comprehensive study of fourteen inbred strains by Southwick and Clark³ revealed no consistent interstrain relationship between fighting and tail rattling. These displays are usually regarded by ethologists as having complex, rather than unitary, motivational determinants⁴. If this is correct, a linear relationship with a single variable, such as attack behaviour, would be unlikely to prove robust. An additional complication arises when genetic differences between subjects are important. The degree of manifestation or expressivity of the display, in relation to the motivational states which underlie it, may be expected to exhibit genetic variation.

In view of the potential complexity of the processes involved, it is clearly imperative that a simplified experimental paradigm is developed, which allows controlled presentation of relevant stimuli and elimination of uncontrolled behavioural feedbacks. Electric shock has proved quite useful for the study of aggression because it is capable of inducing high rates of aggressive responding between paired subjects⁵. Further simplification is possible in the mouse because many of the behaviours observed in aggressive encounters can be elicited by means of footshock applied to a single subject. I undertook the present study to examine the nature of strain differences in tail rattling elicited by shock, and to assess by means of genetic evidence the relevance of the responses obtained to natural fitness.

Roberts⁶ has suggested that the genetic architecture of behavioural traits may be used to assess the relationship of measures obtained in the laboratory to natural fitness, as traits contributing to fitness should display a large amount of non-additive and little additive genetic variance. A measure of the importance of these components of genetic variance can be obtained by examining a series of crosses between inbred lines. If tail rattling elicited by shock is irrelevant with respect to fitness, the mode of inheritance should be largely additive. If, however, the response to shock is closely related to the disposition to exhibit the trait under natural conditions, and if it is indeed an important social behaviour, then a consistent,

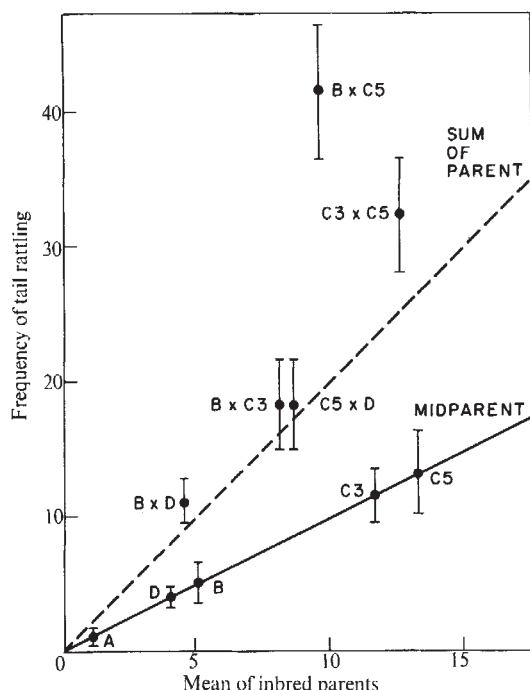


Fig. 1 Frequency of tail rattling of F1 hybrids plotted against the mean of their parent inbred lines. Inbred scores are plotted against themselves and therefore fall on the midparent line (—) defining additive inheritance. All F1's lie above the sum of parent (---), indicating that their scores exceed the sum of the mean scores of the parent inbreds (or twice the midparent values).

largely non-additive (that is, heterotic) mode of inheritance should be displayed.

Five strains of inbred mice (DBA/2Ibg, C57BL/6Ibg, C3H/2Ibg, A/Ibg, BALB/cIbg) and five F1 crosses (C57BL × DBA, DBA × BALB, BALB × C57BL, BALB × C3H, C57BL × C3H) were tested. Reciprocal crosses were included for all five, with the exception of the C57BL × DBA cross in which the female parent was C57BL. Experimental subjects were weaned at 25 days, and littermates of like sex were housed together in 27 × 14 × 10 cm stainless steel cages until testing at 60–80 days of age. Approximately ten male and female mice of each strain were tested, with a maximum of two of each sex from any litter.

Subjects were tested in a 16 × 16 × 13 cm 'Plexiglas' box with a grid floor, illuminated to approximately 158 lux by overhead fluorescent lights. The shock source was a 'Stechman' constant current generator and scrambler. The test consisted of four phases; a series of 10 0.2 mA shocks every 20 s, a 120 s recovery period, a series of 10 0.6 mA shocks, and a final 120 s recovery period. The shock duration was 0.5 s throughout.

The response measure we considered was the total number of bouts of tail rattling exhibited over the four phases of the test session. A bout of tail rattling consisted of a series of rapid lateral undulations in the tail. A number of related behavioural changes were typically associated with tail rattling as it occurred both in response to footshock and in aggressive encounters. Pilo-erection was generally observed together with shivering movements of the dorsum skin. When moving, the mouse often displayed the so-called "mincing gait" in which the animal progresses by short steps with the perineal area pulled forward and pressed against the substrate, and in many cases this was accompanied by sporadic urination. Shivering and peculiar urination pattern accompanying the mincing gait was first noted utilizing shock and was subsequently verified by observation of aggressive encounters.

Because of the pronounced heterogeneity of variance between

strains, a logarithmic transformation was applied to the original data before analysis of variance and range tests. Analysis of variance of the transformed scores indicated the absence of significant sex and reciprocal differences, and sex and reciprocal groups were therefore pooled. Untransformed scores are reported below for simplicity. All hybrids were significantly higher than the mean of the parent strains, and three were significantly higher ($P < 0.05$, t test) than the high parent. Fig. 1 shows the mean scores based on all four test periods of the F1 hybrids plotted against the mean of their parental strains. The inbred strains were plotted against themselves and therefore lie on the midparent line. If the mode of inheritance were essentially additive, the F1 values would also lie on, or close to, this line. The line defining the values which would be assumed if the F1 scores approximated the sum of the inbred parents' scores is also shown, as it emphasizes the extreme degree of heterosis observed. Of the twenty possible comparisons utilizing the four test periods and five crosses, all exceeded the midparent value and eighteen were higher than the high parent.

Table 1 shows the untransformed mean scores of each inbred strain for each period of the test and the per cent of the total number of responses occurring in each period. The horizontal lines separate strains whose log transformed mean scores for the whole test were significantly different at the 0.05 level using Duncan's multiple range test.

The pattern of responding over the four periods of the test reveals further differences between the inbred strains. BALB, which exhibited 98% of its responses in the first period, can readily be distinguished from DBA, which showed a more even response distribution. Similarly C57BL can be distinguished from C3H on the basis of scores in the second period. A characteristic response pattern can therefore be associated with each of the five inbred lines. While strain differences in behaviour are commonplace, it is rather unusual to be able to discriminate this well among a set of strains on the basis of one class of responses.

It seems quite likely that the present method will prove useful in the investigation of the behavioural organization of this display. It should be extremely useful for genetic analysis of segregating populations, as phenotypes can be assigned to individuals rather than to pairs of combatants. While additional experiments will be necessary for extrapolation from shock induced tail rattling to that which occurs in nature, the heterotic mode of inheritance provides some assurance that the method taps psychological mechanisms related to natural fitness. Finally, the diversity in performance encountered among these genotypes is sufficiently extreme and complex that they may perhaps be used as a model system for the study of the evolutionary development of signal function.

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Table 1 Comparison of Tail Rattling among Inbred Lines

Strain		Period				Total score Mean ± s.e.
		I Low shock	II Shock free	III High shock	IV Shock free	
A	Mean	0.78	0.11	0.17	0.06	1.11 ± 0.35
	(%)	(70)	(9)	(15)	(5)	
DBA	Mean	1.25	0.20	1.90*	0.60	3.95 ± 0.74
	(%)	(31)	(5)	(48)	(15)	
BALB	Mean	4.80	0.05	0.05*	0.00	4.90 ± 1.54
	(%)	(98)	(1)	(1)	(0)	
C3H	Mean	4.76	1.62*	3.95	1.43	11.76 ± 2.2
	(%)	(41)	(14)	(34)	(12)	
C57BL	Mean	3.80	4.65*	3.05	1.70	13.20 ± 2.9
	(%)	(29)	(35)	(23)	(13)	

* The BALB strain scored significantly lower ($P < 0.05$) than the DBA strain in period III. The C3H strain scored significantly lower than C57BL in period II.

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UK 786 Possible Species-Specific Toxicant for Norway Rats

UK 786¹ (5-(N-piperidino)-10,11-dihydro-5H-dibenzo (a,d)-cycloheptene (Dr J. Morrison, Pfizer Ltd.)) is a compound whose acute oral LD₅₀ has been reported to be 6.6 mg kg⁻¹ in male albino rats and 1,160 mg in male albino mice². These data suggested that UK 786 might be a highly specific toxicant for rats, and we conducted the following series of tests with thirteen species of birds and mammals to investigate this.

In acute oral toxicity tests, single doses of UK 786 were administered by stomach tube. Propylene glycol solutions were used for red-winged blackbirds, and the animals observed for seven days post treatment, but for all other animals, the chemical was prepared in 0.5% gum tragacanth and the observation period was 14 days. LD₅₀'s with 95% confidence limits were calculated by the methods of Litchfield and Wilcoxon³ (for wild and albino Norway rats and albino mice) or Thompson⁴ and Thompson and Weil⁵ (for Polynesian rats and house sparrows). Sprague-Dawley albino rats, Swiss-Webster albino mice, and domestic rabbits were purchased from commercial suppliers, gerbils and bobwhite quail were from our laboratory colonies, and all other animals were wild-trapped. All animals were adults and were individually caged, except the pigeons, which were caged together.

The acute oral toxicity tests indicated that of the thirteen species tested, only wild and albino Norway rats were highly susceptible to the lethal action of UK 786 (Table 1). Symptoms of poisoning in Norway rats included laboured breathing and cyanosis of the extremities, and death usually

Table 1 Acute Oral Toxicity of UK 786 in Animals

Common name and species	No. and sex tested	Dose (mg kg ⁻¹)	No. died	LD ₅₀ and 95% confidence limits
Bobwhite quail (<i>Colinus virginianus</i>)	2M, 2F	100	0	
Pigeon (<i>Columba livia</i>)	4*	500	0†	
Starling (<i>Sturnus vulgaris</i>)	2M, 2F	500	0	
Red-winged blackbird (<i>Agelaius phoeniceus</i>)	2M	100	0	
House sparrow (<i>Passer domesticus</i>)	6M 2F	— 500	— 0	354 (—)
Rabbit, domestic (<i>Oryctolagus cuniculus</i>)	3M 2M	500 100	1 0	
Deer mouse (<i>Peromyscus maniculatus</i>)	4M 4F	1,600 1,600	1 1	
Gerbil (<i>Meriones unguiculatus</i>)	2M, 2F	500	0	
Rat, ricefield (<i>Rattus r. mindanensis</i>)	8F	7.5, 15, 30, 120	0	
Rat, wild Norway (<i>R. norvegicus</i>)	12M 12F	— —	— —	8.4 (5.3–13.4) 8.9 (4.4–18.2)
Rat, albino (<i>R. norvegicus</i>)	16M	—	—	11.9 (5.7–24.5)
Rat, Polynesian (<i>R. exulans</i>)	6M	—	—	354 (—)
Mouse, albino (<i>Mus musculus</i>)	40M	—	—	205 (85.4–492)

* Sex not determined.

† Three of the four birds regurgitated after dosing.

occurred in 1 to 4 h. Gross pathological examination revealed massive haemorrhaging within the lungs. In other animals that succumbed to UK 786, death occurred two to six days after dosing and no typical symptoms could be identified.

Ricefield, Polynesian, and wild Norway rats were used in voluntary feeding tests with treated bait, made by mixing UK 786 and ground commercial rat chow, and forming the mixture into 105–110 mg tablets. Ricefield and Polynesian rats were each offered one cup containing 10 g of treated tablets for one night. Norway rats were first offered two cups each containing about 20 g of untreated tablets for two consecutive nights; consumption from each cup was recorded daily, and on the third night 20 g of treated tablets were placed in the "preferred" cup and 20 g of untreated tablets in the second cup. Treated tablets were offered for two to four consecutive nights; on subsequent test nights, positions of the treated and untreated cups were alternated.

Table 2 Consumption and Mortality when Food Tablets Containing UK 786 were Offered to Individually Caged Rats for One Night

Rat species	Concentration of UK 786 (%, w/w)	No. and sex tested	Amount eaten				Mortality No.
			Treated food (g)		UK 786 (mg kg ⁻¹)		
			Mean	Range	Mean	Range	
Ricefield *	0.125	4 F	9.9	9.5 –10.0	61.6	52.4– 69.8	0
		5 M	10.0†		56.5	47.3– 69.6	0
	0.25	5 F	4.9	1.9 – 7.4	75.2	30.0–122	0
		5 F	3.5	2.1 – 6.0	320	189 –468	0
Polynesian *	0.25	5 F	1.7	1.2 – 2.9	103	55.7–156	0
	2.0	5 F	0.8	0.1 – 2.1	273	12.9–848	0
Wild Norway ‡	0.25	5 F	3.1	0.41– 8.5	36.9	4.6–118	1
		5 M	1.9	0.64– 4.1	21.3	6.8– 45.3	0
	0.5	5 F	2.1	0.67– 3.9	45.1	18.2– 90.0	3
		5 M	2.1	0.33– 3.3	46.8	5.7– 81.8	2
	1.0	5 F	0.8	0.20– 1.4	50.7	12.1– 97.1	2
		2.0	10 F	0.7	0.14– 1.0	56.2	18.3–121
			10 M	0.6	0.03– 1.9	34.5	1.5–112

* Ricefield and Polynesian rats were offered 10 g of treated tablets only.

† Rats consumed all the bait.

‡ Norway rats were offered 20 g each of treated and untreated tablets.

The results of the feeding tests are summarized in Table 2. There was no mortality in ricefield and Polynesian rats offered bait for one night, but acceptance was poor at the higher concentrations. Although Norway rats were offered treated tablets for two to four nights, mortality occurred only the first night; acceptance was also poor at the higher concentrations, but they ate enough 2% bait to cause 80% mortality. After the first night, consumption of treated tablets was highly variable; some animals completely rejected the bait, others ate less than, as much as, or more than the first night.

Public health and agricultural workers have been searching for many years for chemicals for use in Norway rat control programmes that are relatively nontoxic to man, domestic animals and nontarget wildlife. 'Warfarin' has been in the forefront as a rat toxicant, but after the detection of 'Warfarin'-resistant Norway rats in Great Britain⁶, and recently in the United States⁷, the search for new chemicals to replace it has become more important. Norbormide is selectively toxic to rats⁸ but causes "bait shyness". Our primary target species are ricefield and Polynesian rats, but the preliminary data reported here indicate that UK 786 may be a useful rodenticide for Norway rats.

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Migration of Birds over the South Coast of China recorded by Radar

THE opportunity to record bird movements with radar has undoubtedly existed in the Far East, but we know of no published studies of bird migration that utilized radar in Indonesia, Malaysia, Thailand, Vietnam, the Philippine Islands, Taiwan, American Micronesia or even Japan. We report here a radar study of bird migration over Hong Kong (which lies between 22° 9' and 22° 37' N and 113° 52' and 114° 30' E) to stimulate the use of radar to study bird migrations across or between some of the countries mentioned, and also additional studies on the mainland of China.

During April and May 1972, and again from September to December 1972, we used the 10 cm 650 kW Plessey 43S weather radar of the Royal Observatory, Hong Kong, to detect and study the northward spring and southward autumn migrations of birds over the south coast of China, and particularly over areas immediately to the west, south and east of Hong Kong.

Although there were some breaks in the record, observations were made for nearly four months during 1972.

The findings of detailed ornithological analysis of the 16 mm time-lapse films will be published elsewhere later, but some of the results from the period of the spring migration are mentioned here to indicate the interest of extending these studies to other sites in the region.

North-eastward movements of birds were recorded from mid-April to the end of May in the vicinity of Hong Kong (and corresponding south-westward movements were recorded in September and October). While partly overland and partly offshore along the coast, the majority of the bird echoes involved in this spring movement were almost certainly crossing the South China coast after an over-sea flight from at least as far away as the Chinese island of Hainan (250 miles) and perhaps from as far away as Vietnam. The character and behaviour of the echoes indicated that this movement was mainly of charadriiform species; indeed more than 20 species of shorebirds (waders) were observed in Hong Kong during the period from April 9 to May 7, 1972—the three most abundant species being Redshank, *Tringa totanus*, Eastern Little Stint, *Calidris ruficollis*, and Greater Sand Plover, *Charadrius leschenaultii*.

A most prominent ascent of bird echoes was detected after sunset on at least seven evenings in April offshore over Lema Channel and the Pearl River estuary, the identity of which is uncertain—but perhaps due to terns.

During the period April 24–28, 1972, echoes were recorded apparently arriving at the south coast of China from over the South China Sea, on at least four afternoons, on tracks that ranged from NNW to NNE with a predominance of NNW–N. Using the RHI facility of the 43S radar, we were able to determine that these arriving migrants were flying at altitudes up to a ceiling of 11,000 foot (about 3,700 m). On three evenings in this same period we also observed an onward departure inland in the same northward direction both from part of Hong Kong and also from some Chinese islands a little to the south of Hong Kong. The character of the echoes was of the "passerine-type" during their mass evening departure after sunset. Together, the observation of an arrival from the south and a closely-related onward movement northwards in the same direction indicates a crossing of the South China Sea of between 700 and 1,200 miles to the mainland of China from an origin in the southern Philippine Islands (perhaps Palawan Island) or from the north coast of the island of Borneo, and a non-stop flight for 36–48 h, by an unidentified population of birds that may have over-wintered even further south.

It is thus clear from our preliminary analysis of the records that radar studies of bird migration in other Far Eastern countries (which together compose a network of offshore "barrier archipelagos" and "inland seas") would be very revealing. Furthermore, films made during such studies would dramatically highlight the urgent need for cooperative inter-governmental measures to conserve a migratory bird fauna that, by virtue of its migrations, belongs equally to the several countries of the region from Australasia and Indonesia and Malaysia on the one hand, to China, Japan and the USSR on the other.

We thank Mr G. J. Bell, director of the Royal Observatory, for his enthusiastic interest in determining early in April 1972 whether bird migration was in fact being detected by this equipment, and subsequently in providing facilities for recording the migratory movements by means of time-lapse photography of the radar plan position indicator.

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BOOK REVIEWS

Psychology with a Heart

Psychology is About People. By H. J. Eysenck. Pp. xvii+331. (Allen Lane: London, June 1972.) £3.50.

SOMETHING is stirring in the psychological world: there is a spate of books, broadcasts, and papers which do not deal with technical and detailed issues. Rather they discuss broad questions of principle, perhaps like Professor Hudson in a broadcast attacking "the tyranny of fact" in academic psychology since the war; or perhaps like B. F. Skinner writing a controversial book to argue that an objective and experimental approach is the only way to design a viable culture. Professor Eysenck's book is another contribution of this kind, nearer to Skinner than to Hudson in his views, but nevertheless sharing with Hudson an immediately intelligible and attractive style.

The general thesis of the book is that a hard headed experimental approach is the right one in psychology, but that it is a fallacy to expect the same treatment to produce the same results on all human beings. Rather, Professor Eysenck makes (as he always has done) personality his central variable. The method of argument is to take a number of specific fields: starting with the familiar criticism of psychotherapy as unsupported by objective evidence, moving through Kinsey-like enquiries about sexual behaviour as a function of personality, and leading to a brief popular statement of the claims of behaviour therapy and teaching machines. There is then an argument for the use of intelligence tests and of taking genetic factors into account in education, and a rather wicked discussion of political attitudes as a function of class. Next comes a discussion of pornography and permissiveness in the mass media and finally a lengthy plea for the objective approach on moral grounds.

The strength and weakness of the book can be best illustrated from the section on pornography, which is perhaps the most novel to those who know Professor Eysenck's earlier views. He draws attention to the widespread use by modern therapists of "desensitization" techniques in which a formerly frightening experience is gradually made acceptable by repeated exposure at a safe distance and in a harmless form. This method will sometimes cure irrational phobias: but, he argues,

is it not analogous to the repeated presentation of scenes of violence in the comfort of a living room? Might we not explain in this way why "people ask wonderingly why there has been such a terrible outbreak of violence in the USA"?

This kind of point certainly needs discussion. To argue that human behaviour stems entirely from within and is unaffected by cultural experiences would be to deny almost every finding of social psychology. In any consistent culture there ought therefore to be, and in fact are, limits upon the things that can be said or shown without massive disapproval: our society does not in fact tolerate overt racist statements, nor (if they are too blatant) attempts to whip up hatred against particular religions, contempt for women, and so on. What appears on the mass media ought therefore to be considered, to see whether it is going to produce good changes in society or bad ones: for example, whether it might reduce freedom to express divergent points of view.

But Professor Eysenck's argument passes hastily over difficulties. Desensitization frequently fails in the clinic: and we don't really know why. Is it not therefore even more likely to fail in the broader range of experiences met in the living room? Again, even if we admit the applicability of desensitization, we have the problem of identifying the stimuli most likely to produce it. Is a contemporary film with an X certificate really more desensitizing than a traditional cowboy in which blows produce no blood, bullets no mess of skull and brains, and everybody dies decorously and with dignity? Bluntly, has the United States suffered an increase of violence?

These doubts are general throughout the book: things which are true and badly need saying are mixed with things which are uncertain and might be true or false: and occasionally with statements which most other psychologists would regard as very unlikely. No note of increasing doubt appears as one goes from one to another. Perhaps this is inevitable in a book for broad audiences, but it is bound to worry specialists and to make the book slightly dangerous to the general reader. For the latter it ought to appeal by being easy to read: although for the more strait-laced, its impact may be marred by Professor Eysenck's inability to resist

a joke. Was it really necessary, for an example of the difficulties of experiment, to describe the procedures for establishing the potency of Prince Vincenzo Gonzaga? Yet there are some instances where Professor Eysenck's taste is impeccable: for example, his choice of the career of a great black scientist, Moses Carver, as a counter-example to those who feel that environment determines educational attainment, and his account of the suicide of a homosexual denied behaviour therapy, as a rebuke to those who regard such therapy as unethical. Passages of this kind convince one that Professor Eysenck's heart is in the right place, and this may do more than any of his technical arguments to win supporters for an approach to human beings based on evidence rather than emotion.

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Fisher's Papers

Collected Papers of R. A. Fisher. Edited by J. H. Bennett. Volume I. 1912-24. Pp. 604. (The University of Adelaide: Adelaide, 1971.) A\$17; US\$20.

WHAT is achieved by republication of the complete writings of a distinguished scientist? Not, surely, any immediate addition to his science, for what is clearly important in his work will long since have been assimilated to the general body of knowledge. Yet the opportunity of re-reading the original papers instead of subsequent paraphrases by others, of seeing perhaps for the first time work no longer readily accessible, and of tracing the development of major lines of thought can be rewarding and may influence substantially a new generation.

When the author is one who both created, almost single-handed, one field of science and gave much to another, the importance of such publication is incalculable. Ten years after R. A. Fisher died, the University of Adelaide begins a five-volume collection of all his main publications and a few short reviews. These are reproduced photographically from the originals, enlarged or reduced to a standard page size, thus preserving their individuality and an appearance familiar to many. Where available, additional notes and comments by Fisher are added; the editor, Professor

J. H. Bennett, has also incorporated many numerical and typographical corrections as footnotes.

The first volume contains forty-one papers published before 1925. Although during this period statistical theory predominated, Fisher had already manifested a lifelong concern for eugenics and this had stimulated him to make major advances in genetics. The earliest paper, that of 1912 in which he already stated the idea of estimation by maximum likelihood, is an appropriate pointer to a life spent in seeking the optimal use of information. Twenty years later he could assert confidently that "When a biologist believes there is information in an observation, it is up to the statistician to get it out". This volume, of course, includes the great *Philosophical Transactions* paper and others that so firmly established the theory of statistical inference. Here also the study of exact sampling distributions is taken as far as the various beta and gamma types deriving from the normal distribution. Already Fisher had clarified the analysis of contingency tables through specification of degrees of freedom, and had achieved the simplifications in regression theory basic to so much statistical practice today; the papers are now little known but well deserve attention. Yet again, this period includes the beginnings of the analysis of variance. From the need to interpret the classical Rothamsted experiments, Fisher first devised logical instruments for extracting and organizing the information, then saw how this led to new concepts of experimental design; this series of papers still deserves to be read as a counterbalance to the abstract approach so common today.

Though this giant among statisticians was but one among many great figures in genetics, his contribution to genetics was distinctive, important, and influential. Among these early papers appear some of his first steps to reconcile Darwinism and Mendelism; later he was to do much to show the complementarity of the two. Already he showed his interest both in frequencies at and re-combinations between major gene loci and in the biometric genetics of characters dependent upon summed effects of many factors. The mastery with which this young man of 28, a mathematician untrained in biology, could write "On the Correlation Between Relatives" was a foretaste of the mark he was to make on quantitative genetics; republication of this frequently quoted paper is particularly welcome. The more specifically eugenic papers may now have little scientific value but they throw light on the man; they illustrate the power with which he wrote, his attention both to detail and to grand design, and his care for

the choice of precise word and telling phrase.

This volume is attractive in appearance, pleasant to handle and read, and a credit to both editor and publisher.

D. J. FINNEY

Disks of *Drosophila*

The Biology of Imaginal Disks. Edited by H. Ursprung and R. Nothiger. Pp. xvii+172. (Springer: Berlin and New York, 1972.) 46 DM; \$14.60.

PROFESSOR HADORN's 70th birthday was preceded by a great deal of surreptitious arrangements with publishers. One of the results is a celebratory volume *The Biology of Imaginal Disks*. It is, therefore, a good moment to consider the status of research in this field, and what general problems it can hope to illuminate in the future.

Up to now, studies on disks have passed through three major periods. In the first era, transplantation, dissociation and reaggregation techniques were used to construct fate maps and to investigate the determined state of different regions of the disks. In the second period the maintenance of the determined state was studied. Here two main discoveries were made, one being the great stability of cell type stemming from even small parts of disks transferred from insect to insect and undergoing hundreds of cell divisions. The other, that at a frequency much higher than that for a typical mutation rate, the cells transformed into cells of another disk (transdetermination). Some transdeterminations turned out to be more probable than others. The third major area, still under active investigation now, is the use of genetic mosaics to analyse those cell interactions which lead to pattern formation in the disks. This method has established that determination and transdetermination are phenomena that occur simultaneously in a group of adjacent cells, which do not themselves constitute a clone.

Currently there are signs of a new developmental genetics analysing visible mutants and lethals by techniques combining clonal analysis with experimental transplantations. The main advantage here is that somatic crossing over can be triggered by X-irradiation at any stage of development chosen by the investigator.

All these matters are well discussed in the book. In addition Fristrom and Oberlander report the development of suitable *in vitro* methods and isolation procedures to do biochemistry on disks. It may be too much to hope for some elucidation of the biochemical basis of determination, but there is more cause for optimism with this system than with any other I can think of.

The main appeal of the book is that

it is very much alive, squarely set in the present time and conveying the excitement in the field. Even if you do not read it until next year it will be more up to date than most books published in 1973. Imaginal disks are a model system for developmental biologists who should find the book fascinating and challenging. Happy birthday, Professor Hadorn.

P. A. LAWRENCE

Idiosyncratic Astrophysics

Dimensional Analysis and Group Theory in Astrophysics. By Rudolf Kurth. Pp. xiv+235. (Pergamon: Oxford and New York, July 1972.) £7.50.

THIS somewhat idiosyncratic book is interesting but hardly essential reading for workers in the field. In his preface, the author engagingly admits that he had originally envisaged group theory as an underlying theme but that its role in dimensional analysis subsequently appeared less significant. Consequently he has devoted two-thirds of the book to dimensional analysis, with applications to stellar radiation and structure, and the remaining third to reduction of group-invariant systems of differential equations, with application to stellar structure. Examples concerning planets appear in three appendices.

Dimensional analysis is widely used in some branches of physics, but astrophysicists' main use of it seems to be in introductory courses, to give estimates of the order of magnitude of quantities like stellar central temperature. Kurth, however, is trying in this book to make much more serious and extensive use of the technique, and meets with reasonable success. Most of the applications he describes are his own original work. The section on group theory neatly links some well-known methods but contains less that is new.

Kurth's exposition is generally clear and concise, sometimes admirably so, but he occasionally comes irritatingly close to insulting the reader's intelligence. Only once did I find him incomprehensible.

A few specific points should perhaps be recorded. The introductory discussion of the three classical tests of relativity seemed to me top-heavy with assumptions. No reason is given why units of derived quantities are postulated to be monomials of the basic units (which, for example, excludes use of magnitude rather than luminosity). The discussion of the *a priori* probability distribution function for the constants of proportionality is somewhat unsatisfactory, since the criteria for the choice of such a distribution are not examined.

M. A. H. MACCALLUM